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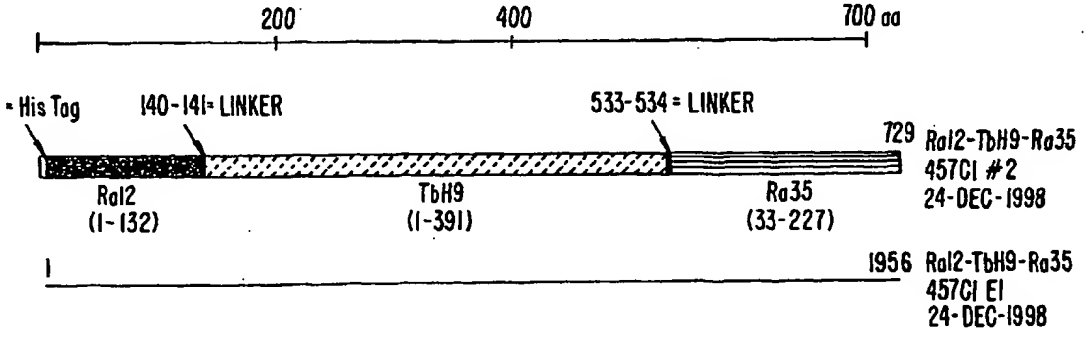
(54) Title: FUSION PROTEINS OF MYCOBACTERIUM TUBERCULOSIS



(57) Abstract: The present invention relates to compositions and fusion proteins containing at least two *Mycobacterium* sp. antigens, and nucleic acids encoding such compositions and fusion proteins. The compositions of the invention increase serological sensitivity of sera from individuals infected with tuberculosis, and methods for their use in the diagnosis, treatment, and prevention of tuberculosis infection.

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FUSION PROTEINS OF MYCOBACTERIUM TUBERCULOSIS

CROSS-REFERENCES TO RELATED APPLICATIONS

: FUSION PROTEINS OF MYCOBACTERIUM TUBERCULOSIS [.S. patent application No. 09/597,796, filed June 20, 2000, and U.S. patent application No. 60/265,737, filed February 1, 2001, herein each incorporated by reference in their entirety.

The present application is related to U.S. patent application No. 09/056,556, filed April 7, 1998; U.S. patent application No. 09/223,040, filed December 30, 1998; U.S. patent application No. 09/287,849, filed April 7, 1999; published PCT application No. WO99/51748, filed April 7, 1999 (PCT/US99/07717), U.S. patent application No. 60/158,338, filed October 7, 1999, and U.S. application No. 60/158,425, filed October 7, 1999; U.S. application No. 09/688,672, filed October 10, 2000; and published PCT application No. WO01/24820, filed October 10, 2000 (PCT/US00/28095); herein each incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable.

FIELD OF THE INVENTION

The present invention relates to fusion proteins containing at least two *Mycobacterium* sp. antigens. In particular, it relates to nucleic acids encoding fusion proteins that include two or more individual *M. tuberculosis* antigens, which increase serological sensitivity of sera from individuals infected with tuberculosis, and methods for their use in the diagnosis, treatment, and prevention of tuberculosis infection.

BACKGROUND OF THE INVENTION

Tuberculosis is a chronic infectious disease caused by infection with *M. tuberculosis* and other *Mycobacterium* species. It is a major disease in developing countries, as well as an increasing problem in developed areas of the world, with about 8 million new cases and 3 million deaths each year. Although the infection may be asymptomatic for a considerable period of time, the disease is most commonly manifested

as an acute inflammation of the lungs, resulting in fever and a nonproductive cough. If untreated, serious complications and death typically result.

Although tuberculosis can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease. Infected individuals may be asymptomatic, but contagious, for some time. In addition, although compliance with the treatment regimen is critical, patient behavior is difficult to monitor. Some patients do not complete the course of treatment, which can lead to ineffective treatment and the development of drug resistance.

In order to control the spread of tuberculosis, effective vaccination and accurate early diagnosis of the disease are of utmost importance. Currently, vaccination with live bacteria is the most efficient method for inducing protective immunity. The most common mycobacterium employed for this purpose is *Bacillus* Calmette-Guerin (BCG), an avirulent strain of *M. bovis*. However, the safety and efficacy of BCG is a source of controversy and some countries, such as the United States, do not vaccinate the general public with this agent.

Diagnosis of tuberculosis is commonly achieved using a skin test, which involves intradermal exposure to tuberculin PPD (protein-purified derivative). Antigen-specific T cell responses result in measurable induration at the injection site by 48-72 hours after injection, which indicates exposure to mycobacterial antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot be distinguished from infected individuals.

While macrophages have been shown to act as the principal effectors of *Mycobacterium* immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against *Mycobacterium* infection is illustrated by the frequent occurrence of *Mycobacterium* infection in AIDS patients, due to the depletion of $CD4^+$ T cells associated with human immunodeficiency virus (HIV) infection. *Mycobacterium*-reactive $CD4^+$ T cells have been shown to be potent producers of γ - interferon (IFN-γ), which, in turn, has been shown to trigger the anti-mycobacterial effects of macrophages in mice. While the role of IFN-γ in humans is less clear, studies have shown that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-γ or tumor necrosis factor-alpha, activates human macrophages to inhibit *M. tuberculosis* infection. Furthermore, it is known that IFN-γ stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, interleukin-12 (IL-12) has been shown to play a role in stimulating resistance to *M. tuberculosis* infection. For a review of the

immunology of *M. tuberculosis* infection, *see* Chan & Kaufmann, *Tuberculosis: Pathogenesis, Protection and Control* (Bloom ed., 1994), and *Harrison's Principles of Internal Medicine*, volume 1, pp. 1004-1014 and 1019-1023 (14th ed., Fauci *et al.*, eds., 1998).

Accordingly, there is a need for improved diagnostic reagents, and improved methods for diagnosis, preventing and treating tuberculosis.

SUMMARY OF THE INVENTION

The present invention therefore provides compositions comprising at least two heterologous antigens, fusion proteins comprising the antigens, and nucleic acids encoding the antigens, where the antigens are from a *Mycobacterium* species from the tuberculosis complex and other *Mycobacterium* species that cause opportunistic infections in immune compromised patients. The present invention also relates methods of using the polypeptides and polynucleotides in the diagnosis, treatment and prevention of *Mycobacterium* infection.

In one aspect, the present invention provides compositions and fusion proteins comprising a mutated version of Ra35 (N-terminal portion of MTB32A) or Ra35FL (full length MTB32A), in which one, two, or three of the three amino acids histidine, aspartate, or serine at the active site has been mutated to a different amino acid. In one embodiment, in Ra35FL, the serine at position 183 has been mutated to an alanine residue, creating Ra35FLMutSA. In one embodiment, the DNA encoding Ra35FL has been mutated by changing a T to a G, resulting in a serine to alanine mutation at amino acid 183 of SEQ ID NO:4. In another embodiment, the present invention provides the fusion protein MTB72FMutSA, in which the Ra35 component of the fusion protein has a serine to alanine mutation at amino acid position 710 of the MTB72F sequence. In another embodiment, the present invention provides a nucleic acid encoding the fusion protein MTB72F, in which the nucleic acid encoding the Ra35 component has been mutated by changing a T to a G, resulting in a serine to alanine mutation at amino acid position 710 of the MTB72F sequence.

The present invention is based, in part, on the inventors' discovery that fusion polynucleotides, fusion polypeptides, or compositions that contain at least two heterologous *M. tuberculosis* coding sequences or antigens are highly antigenic and upon administration to a patient increase the sensitivity of tuberculosis sera. In addition, the

compositions, fusion polypeptides and polynucleotides are useful as diagnostic tools in patients that may have been infected with *Mycobacterium*.

In one aspect, the compositions, fusion polypeptides, and nucleic acids of the invention are used in *in vitro* and *in vivo* assays for detecting humoral antibodies or cell-mediated immunity against *M. tuberculosis* for diagnosis of infection or monitoring of disease progression. For example, the polypeptides may be used as an *in vivo* diagnostic agent in the form of an intradermal skin test. The polypeptides may also be used in *in vitro* tests such as an ELISA with patient serum. Alternatively, the nucleic acids, the compositions, and the fusion polypeptides may be used to raise anti-*M. tuberculosis* antibodies in a non-human animal. The antibodies can be used to detect the target antigens *in vivo* and *in vitro*.

In another aspect, the compositions, fusion polypeptides and nucleic acids may be used as immunogens to generate or elicit a protective immune response in a patient. The isolated or purified polynucleotides are used to produce recombinant fusion polypeptide antigens *in vitro*, which are then administered as a vaccine. Alternatively, the polynucleotides may be administered directly into a subject as DNA vaccines to cause antigen expression in the subject, and the subsequent induction of an anti-*M. tuberculosis* immune response. Thus, the isolated or purified *M. tuberculosis* polypeptides and nucleic acids of the invention may be formulated as pharmaceutical compositions for administration into a subject in the prevention and/or treatment of *M. tuberculosis* infection. The immunogenicity of the fusion protein or antigens may be enhanced by the inclusion of an adjuvant, as well as additional fusion polypeptides, from *Mycobacterium* or other organisms, such as bacterial, viral, mammalian polypeptides. Additional polypeptides may also be included in the compositions, either linked or unlinked to the fusion polypeptide or compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows percent survival of Guinea pigs vaccinated with MTB72F polyprotein.

Figure 2 shows CFUs from spleen cells (Fig. 2A) and lung cells after immunization with MTB72F, MTB59F, MTB72F DNA, or a composition comprising Ra12, TbH9, and Ra35 antigens.

Figure 3 shows a schematic diagram of MTB72F.

Figure 4 shows the nucleotide and amino acid sequence of Ra35 (195 amino acids from the N-terminal portion of MTB32A).

Figure 5 shows an alignment of the amino acid sequences of MTB72F and the mutated version MTB72FMutSA.

Figure 6 shows an alignment of the amino acid sequences of mature (full length) Ra35/MTB32A and the mutated version Ra35FLMutSA.

Figure 7 shows long term survival of guinea pigs vaccinated with Mtb72F formulations.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention relates to compositions comprising antigen compositions and fusion polypeptides useful for the diagnosis and treatment of *Mycobacterium* infection, polynucleotides encoding such antigens, and methods for their use. The antigens of the present invention are polypeptides or fusion polypeptides of *Mycobacterium* antigens and immunogenic thereof. More specifically, the compositions of the present invention comprise at least two heterologous polypeptides of a *Mycobacterium* species of the tuberculosis complex, e.g., a species such as *M. tuberculosis, M. bovis,* or *M. africanum,* or a *Mycobacterium* species that is environmental or opportunistic and that causes opportunistic infections such as lung infections in immune compromised hosts (e.g., patients with AIDS), e.g., *BCG, M. avium, M. intracellulare, M. celatum, M. genavense, M. haemophilum, M. kansasii, M. simiae,, M. vaccae, M. fortuitum,* and *M. scrofulaceum (see, e.g., Harrison's Principles of Internal Medicine*, volume 1, pp. 1004-1014 and 1019-1023 (14th ed., Fauci *et al.*, eds., 1998). The inventors of the present application surprisingly discovered that compositions and fusion proteins comprising at least two heterologous *Mycobacterium* antigens, or immunogenic fragments thereof, where highly antigenic. These compositions, fusion polypeptides, and the nucleic acids that encode them are therefore useful for eliciting protective response in patients, and for diagnostic applications.

The antigens of the present invention may further comprise other components designed to enhance the antigenicity of the antigens or to improve these antigens in other aspects, for example, the isolation of these antigens through addition of a stretch of histidine residues at one end of the antigen. The compositions, fusion polypeptides, and nucleic acids of the invention can comprise additional copies of antigens, or additional heterologous polypeptides from *Mycobacterium* sp., such as

MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen, MTB40 antigen, MTB41 antigen, 38-1, TbRa3, 38 kD, DPEP, TbH4, DPPD, ESAT-6 antigen, MTB85 complex antigen (e.g., MTB85b), or α-crystalline antigen, and Erd14. The compositions, fusion polypeptides, and nucleic acids of the invention can also comprise additional heterologous polypeptides from other non-*Mycobacterium* sources. For example, the compositions and fusion proteins of the invention can include polypeptides or nucleic acids encoding polypeptides, wherein the polypeptide enhances expression of the antigen, e.g., NS1, an influenza virus protein, or an immunogenic portion thereof (*see, e.g.* WO99/40188 and WO93/04175). The nucleic acids of the invention can be engineered based on codon preference in a species of choice, e.g., humans.

The compositions of the invention can be naked DNA, or the compositions, e.g., polypeptides can also comprise adjuvants, e.g., MPL, 3D-MPL, IFA, AS adjuvants such as AS2, AS2', AS2'', AS4, AS6, ENHANZYN (Detox), QS21, CWS, TDM, AGP, CPG, Leif, saponin, and saponin mimetics, and derivatives thereof. In addition, the compositions of the invention can comprise BCG or Pvac as an adjuvant.

In one embodiment, the compositions and fusion proteins of the invention are composed of at least two antigens selected from the group consisting of a MTB39 antigen or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a MTB32A antigen or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

In another embodiment, the antigens are selected from the group consisting of a MTB39 antigen or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a polypeptide comprising at least 205 amino acids of the N-terminus of a MTB32A antigen from a *Mycobacterium* species of the tuberculosis complex.

In another embodiment, the antigens are selected from the group consisting of a MTB39 antigen or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, a polypeptide comprising at least about 205 amino acids of the N-terminus of a MTB32A antigen from a *Mycobacterium* species of the tuberculosis complex, and a polypeptide comprising at least about 132 amino acids from the C-terminus of MTB32A antigen from a *Mycobacterium* species of the tuberculosis complex.

In the nomenclature of the application, Ra35 refers to the N-terminus of MTB32A (Ra35FL), comprising at least about 195 to 205 amino acids of MTB32A from

M. tuberculosis, or the corresponding region from another *Mycobacterium* species. Ra12 refers to the C-terminus of MTB32A (Ra35FL), comprising at least about the last 132 amino acids from MTB32A from *M. tuberculosis*, or the corresponding region from another *Mycobacterium* species.

The following provides sequences of some antigens used in the compositions and fusion proteins of the invention:

SEQ ID NO:1-4: MTB32A (Ra35FL or Ra35 mature), the sequence of which is also disclosed as SEQ ID NO:17 (cDNA) and SEQ ID NO:79 (protein) in the U.S. patent applications No. 08/523,436, 08/523,435, No. 08/658,800, No. 08/659,683, No. 08/818,112, No. 09/056,556, and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications, *see also* Skeiky *et al., Infection and Immunity* 67:3998-4007 (1999). The term MTB32A also includes MTB32A amino acid sequences in which any one of the three amino acids at the active site triad (His, Asp, Ser), e.g., the serine residue at amino acid position 207 in SEQ ID NO:2 or amino acid position 183 in SEQ ID NO:4, has been changed to another amino acid (e.g., alanine, Ra35FLMutSA, *see, e.g.*, Figure 6 and SEQ ID NO:6).

SEQ ID NO:5 and 6: Ra35FLMut SA, the mature version of RA35FL in which the serine residue at amino acid position 183 of SEQ ID NO:4 has been changed to an alanine residue.

SEQ ID NO:7 and 8: Ra35, the N-terminus of MTB32A (Ra35FL), comprising at least about 195 amino acids from the N-terminus of MTB32A from *M. tuberculosis*, the nucleotide and amino acid sequence of which is disclosed in Figure 4 (see also amino acids 33-227 of SEQ ID NO:2 and amino acids 8 to 202 of SEQ ID NO:4). The term Ra35 (N-term) also includes Ra35 amino acid sequences in which any one of the three amino acids at the active site triad (i.e., His, Asp, or Ser) has been changed as described above.

SEQ ID NO:9 and 10: MTBRa12, the C-terminus of MTB32A (Ra35FL), comprising at least about 132 amino acids from the C-terminus of MTB32A from *M. tuberculosis* (*see, e.g.*, amino acids 224 to 355 of SEQ ID NO:2 and amino acids 199 to 330 of SEQ ID NO:4), the sequence of which is disclosed as SEQ ID NO:4 (DNA) and SEQ ID NO:66 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967.

SEQ ID NO:11, 12, 13, and 14: MTB39 (TbH9), the sequence of which is disclosed as SEQ ID NO:106 (cDNA full length) and SEQ ID NO:107 (protein full length) in the U.S. patent applications No. 08/658,800, No. 08/659,683, No. 08/818,112, and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications. The sequence is also disclosed as SEQ ID NO:33 (DNA) and SEQ ID NO:91 (amino acid) in U.S. patent application No. 09/056,559.

The following provides sequences of some fusion proteins of the invention

SEQ ID NO:15 and 16: MTB72F (Ra12-TbH9-Ra35), the sequence of which is disclosed as SEQ ID NO:1 (DNA) and SEQ ID NO:2 (protein) in the US patent application No. 09/223,040, No. 09/223,040, and in the PCT/US99/07717 application. The term MTB372F also includes MTB72F amino acid sequences in which any one of the three amino acids at the active site triad in Ra35FL (i.e., His, Asp, or Ser), has been changed as described above (*see, e.g.*, MTB72FMutSA, Figure 5).

SEQ ID NO:17 and 18: MTB72FMutSA (Ra12-TbH9-Ra35MutSA), wherein, in the Ra35 component of the fusion protein, the serine at position 710 has been changed to an alanine.

SEQ ID NO:19 and 20: TbH9-Ra35 (MTB59F), the sequence of which is disclosed as SEQ ID NO:23 (cDNA) and SEQ ID NO:24 (protein) in the U.S. patent application No. 09/287,849 and in the PCT/US99/07717 application.

The following provides sequences of some additional antigens used in the compositions and fusion proteins of the invention:

SEQ ID NO: 21 and 22: MTB8.4 (DPV), the sequence of which is disclosed as SEQ ID NO:101 (cDNA) and SEQ ID NO:102 (protein) in the U.S. patent applications No. 08/658,800, No. 08/659,683, No. 08/818,112 and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications.

SEQ ID NO:23 and 24: MTB9.8 (MSL), the sequence of which is disclosed as SEQ ID NO:12 (DNA), SEQ ID NO:109 (predicted amino acid sequence) and SEQ ID NO:110 to 124 (peptides) in the U.S. patent applications No. 08/859,381, No. 08/858,998, No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications.

SEQ ID NO:25, 26, and 27: MTB9.9A (MTI, also known as MTI-A), the sequence of which is disclosed as SEQ ID NO:3 and SEQ ID NO:4 (DNA) and SEQ ID

NO:29 and SEQ ID NO:51 to 66 (ORF peptide for MTI) in the U.S. patent applications No. 08/859,381, No. 08/858,998, No. 09/073,009 and v09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications. Two other MTI variants also exist, called MTI-B and MTI-C.

SEQ ID NO:28 and 29: MTB40 (HTCC#1), the sequence of which is disclosed as SEQ ID NO:137 (cDNA) and 138 (predicted amino acid sequence) in the U.S. patent applications No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications.

SEQ ID NO:30 and 31: MTB41 (MTCC#2), the sequence of which is disclosed as SEQ ID NO:140 (cDNA) and SEQ ID NO:142 (predicted amino acid sequence) in the U.S. patent applications No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications.

SEQ ID NO:32 and 33: ESAT-6, the sequence of which is disclosed as SEQ ID NO:103 (DNA) and SEQ ID NO:104 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967. The sequence of ESAT-6 is also disclosed in U.S. Patent No. 5,955,077.

SEQ ID NO:34 and 35: Tb38-1 or 38-1 (MTb11), the sequence of which is disclosed in SEQ ID NO:46 (DNA) and SEQ ID NO:88 (predicted amino acid) in the U.S. patent application Nos. 09/072,96; 08/523,436; 08/523,435; 08/818,112; and 08/818,111; and in the WO97/09428 and WO97/09429 applications.

SEQ ID NO:36 and 37: TbRa3, the sequence of which is disclosed in SEQ ID NO:15 (DNA) and SEQ ID NO:77 (predicted amino acid sequence) of WO 97/09428 and WO97/09429 applications.

SEQ ID NO:38 and 39: 38 kD, the sequence of which is disclosed in SEQ ID NO:154 (DNA) and SEQ ID NO:155 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967. 38 kD has two alternative forms, with and without the N-terminal cysteine residue.

SEQ ID NO:40 and 41: DPEP, the sequence of which is disclosed in SEQ ID NO:52 (DNA) and SEQ ID NO:53 (predicted amino acid sequence) in the WO97/09428 and WO97/09429 publications.

SEQ ID NO:42 and 43: TbH4, the sequence of which is disclosed as SEQ ID NO:43 (DNA) and SEQ ID NO:81 (predicted amino acid sequence) in WO97/09428 and WO97/09429 publications.

SEQ ID NO:44 and 45: DPPD, the sequence of which is disclosed in SEQ ID NO:240 (DNA) and SEQ ID NO:241 (predicted amino acid sequence) in USSN 09/072,967 and in the PCT/US99/03268 and PCT/US99/03265 applications. The secreted form of DPPD is shown herein in Figure 12 of PCT/US00/28095.

MTb82 (MTb867), the sequence of which is disclosed in Figures 8 (DNA) and 9 (amino acid) of PCT/US00/2809.

Erd14 (MTb16), the cDNA and amino acids sequences of which are disclosed in Verbon *et al., J. Bacteriology* 174:1352-1359 (1992).

α-crystalline antigen, the sequence of which is disclosed in Verbon *et al., J. Bact.* 174:1352-1359 (1992);

85 complex antigen, e.g., 85b antigen, the sequence of which is disclosed in Content *et al., Infect. & Immunol.* 59:3205-3212 (1991).

The following provides sequences of some additional fusion proteins used in the compositions and fusion proteins of the invention:

SEQ ID NO:46 and 47: DPV-MTI-MSL-MTCC#2 (MTb71F), the sequence of which is disclosed as SEQ ID NO:15 (nucleic acid) and in SEQ ID NO:16: (protein) in the U.S. patent application No. 09/287,849 and in the PCT/US99/07717 application.

SEQ ID NO:48 and 49: DPV-MTI-MSL (MTb31F), the sequence of which is disclosed in SEQ ID NO:18 (cDNA) and SEQ ID NO:19 (protein) in the U.S. patent application No. 09/287,849 and in the PCT/US99/07717 application.

Each of the above sequences is also disclosed in Cole *et al. Nature* 393:537 (1998) and can be found at, e.g., http://www.sanger.ac.uk and http:/www.pasteur.fr/mycdb/.

The above sequences are disclosed in U.S. patent applications Nos. 08/523,435, 08/523,436, 08/658,800, 08/659,683, 08/818,111, 08/818,112, 08/942,341, 08/942,578, 08/858,998, 08/859,381, 09/056,556, 09/072,596, 09/072,967, 09/073,009, 09/073,010, 09/223,040, 09/287,849 09/597,796; and in PCT patent applications PCT/US00/28095; PCT/US98/10407, PCT/US98/10514, PCT/US99/03265, PCT/US99/03268, PCT/US99/07717, WO97/09428 and WO97/09429, WO98/16645, WO98/16646, each of which is herein incorporated by reference.

The antigens described herein include polymorphic variants and conservatively modified variations, as well as inter-strain and interspecies *Mycobacterium* homologs. In addition, the antigens described herein include subsequences or truncated sequences. The fusion proteins may also contain additional polypeptides, optionally heterologous peptides from *Mycobacterium* or other sources. These antigens may be modified, for example, by adding linker peptide sequences as described below. These linker peptides may be inserted between one or more polypeptides which make up each of the fusion proteins.

DEFINITIONS

"Fusion polypeptide" or "fusion protein" refers to a protein having at least two heterologous *Mycobacterium* sp. polypeptides covalently linked, either directly or via an amino acid linker. The polypeptides forming the fusion protein are typically linked C-terminus to N-terminus, although they can also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. The polypeptides of the fusion protein can be in any order. This term also refers to conservatively modified variants, polymorphic variants, alleles, mutants, subsequences, interspecies homologs, and immunogenic fragments of the antigens that make up the fusion protein. *Mycobacterium tuberculosis* antigens are described in Cole *et al., Nature* 393:537 (1998), which discloses the entire *Mycobacterium tuberculosis* genome. The complete sequence of *Mycobacterium tuberculosis* can also be found at http://www.sanger.ac.uk and at http://www.pasteur.fr/mycdb/ (MycDB). Antigens from other *Mycobacterium* species that correspond to *M. tuberculosis* antigens can be identified, e.g., using sequence comparison algorithms, as described herein, or other methods known to those of skill in the art, e.g., hybridization assays and antibody binding assays. Fusion proteins of the invention can also comprise additional copies of a component antigen or immunogenic fragment thereof.

A polynucleotide sequence comprising a fusion protein of the invention hybridizes under stringent conditions to at least two nucleotide sequences, each encoding an antigen polypeptide selected from the group consisting of MTB39 or an immunogenic fragment thereof and MTB32A or an immunogenic fragment thereof. The polynucleotide sequences encoding the individual antigens of the fusion polypeptide therefore include conservatively modified variants, polymorphic variants, alleles, mutants, subsequences, immunogenic fragments, and interspecies homologs of MTB39 and MTB32A. The

polynucleotide sequence encoding the individual polypeptides of the fusion protein can be in any order.

In some embodiments, the individual polypeptides of the fusion protein are in order (N- to C- terminus) from large to small. Large antigens are approximately 30 to 150 kD in size, medium antigens are approximately 10 to 30 kD in size, and small antigens are approximately less than 10 kD in size. The sequence encoding the individual polypeptide may be as small as, e.g., an immunogenic fragment such as an individual CTL epitope encoding about 8 to 9 amino acids, or, e.g., an HTL or B cell epitope. The fragment may also include multiple epitopes. The immunogenic fragment may also represent a larger part of the antigen sequence, e.g., about 50% or more of MTB39 and MTB32A, e.g., the N- and C-terminal portions of MTB32A. Preferred immunogenic fragments of MTB32A include Ra12, Ra35, and Ra35 MutSA.

A fusion polypeptide of the invention specifically binds to antibodies raised against at least two antigen polypeptides, wherein each antigen polypeptide is selected from the group consisting of MTB39 or an immunogenic portion or fragment thereof and MTB32A or an immunogenic portion thereof. The antibodies can be polyclonal or monoclonal. Optionally, the fusion polypeptide specifically binds to antibodies raised against the fusion junction of the antigens, which antibodies do not bind to the antigens individually, i.e., when they are not part of a fusion protein. The fusion polypeptides optionally comprise additional polypeptides, e.g., three, four, five, six, or more polypeptides, up to about 25 polypeptides, optionally heterologous polypeptides or repeated homologous polypeptides, fused to the at least two heterologous antigens. The additional polypeptides of the fusion protein are optionally derived from *Mycobacterium* as well as other sources, such as other bacterial, viral, or invertebrate, vertebrate, or mammalian sources. The individual polypeptides of the fusion protein can be in any order. As described herein, the fusion protein can also be linked to other molecules, including additional polypeptides. The compositions of the invention can also comprise additional polypeptides that are unlinked to the fusion proteins of the invention. These additional polypeptides may be heterologous or homologous polypeptides.

The term "fused" refers to the covalent linkage between two polypeptides in a fusion protein. The polypeptides are typically joined via a peptide bond, either directly to each other or via an amino acid linker. Optionally, the peptides can be joined via non-peptide covalent linkages known to those of skill in the art.

"FL" refers to full-length, i.e., a polypeptide that is the same length as the wild-type polypeptide.

The term "immunogenic fragment thereof" refers to a polypeptide comprising an epitope that is recognized by cytotoxic T lymphocytes, helper T lymphocytes or B cells. Preferred immunogenic fragments of, e.g., MTB32A, are RA35, Ra35MutSA, or Ra12.

The term "*Mycobacterium* species of the tuberculosis complex" includes those species traditionally considered as causing the disease tuberculosis, as well as *Mycobacterium* environmental and opportunistic species that cause tuberculosis and lung disease in immune compromised patients, such as patients with AIDS, e.g., *M. tuberculosis, M. bovis,* or *M. africanum, BCG, M. avium, M. intracellulare, M. celatum, M. genavense, M. haemophilum, M. kansasii, M. simiae, M. vaccae, M. fortuitum,* and *M. scrofulaceum (see, e.g., Harrison's Principles of Internal Medicine*, volume 1, pp. 1004-1014 and 1019-1023 (14th ed., Fauci *et al.*, eds., 1998).

An adjuvant refers to the components in a vaccine or therapeutic composition that increase the specific immune response to the antigen (*see, e.g.*, Edelman, *AIDS Res. Hum Retroviruses* 8:1409-1411 (1992)). Adjuvants induce immune responses of the Th1-type and Th-2 type response. Th1-type cytokines (e.g., IFN-γ, IL-2, and IL-12) tend to favor the induction of cell-mediated immune response to an administered antigen, while Th-2 type cytokines (e.g., IL-4, IL-5, Il-6, IL-10 and TNF-β) tend to favor the induction of humoral immune responses.

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in

which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine.

Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)

(*see, e.g.*, Creighton, *Proteins* (1984)).

The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a

coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, optionally 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary

"moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

"Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'$_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'$_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'$_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990)).

For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975);

Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy* (1985)). Techniques for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990); Marks *et al.*, *Biotechnology* 10:779-783 (1992)).

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to fusion proteins can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with fusion protein and not with individual components of the fusion proteins. This selection may be achieved by subtracting out antibodies that cross-react with the individual antigens. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an individual antigen or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not diminished, relative to a fusion polypeptide comprising native antigens. Variants preferably exhibit at least about 70% identity, more preferably

at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native polypeptide or a portion thereof.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 70% identity, optionally 75%, 80%, 85%, 90%, or 95% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to the compliment of a test sequence. Optionally, the identity exists over a region that is at least about 25 to about 50 amino acids or nucleotides in length, or optionally over a region that is 75-100 amino acids or nucleotides in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 25 to 500, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575

Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g., Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. 1995 supplement)).

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, e.g., version 7.0 (Devereaux *et al., Nuc. Acids Res.* 12:387-395 (1984).

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al., Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al., J. Mol. Biol.* 215:403-410 (1990), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al., supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment

score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

POLYNUCLEOTIDE COMPOSITIONS

As used herein, the terms "DNA segment" and "polynucleotide" refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a polypeptide refers to a DNA segment that contains one or more coding sequences yet is substantially isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the terms "DNA segment" and "polynucleotide" are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

As will be understood by those skilled in the art, the DNA segments of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

The terms "isolated," "purified," or "biologically pure" therefore refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Of course, this refers to the DNA segment as originally isolated, and does not exclude other isolated proteins, genes, or coding regions later added to the composition by the hand of man. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. An isolated nucleic acid is separated from other open reading frames that flank the gene and encode proteins other than the gene.

As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes a *Mycobacterium* antigen or a portion thereof) or may comprise a variant, or a biological or antigenic functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. The term "variants" also encompasses homologous genes of xenogenic origin.

In additional embodiments, the present invention provides isolated polynucleotides and polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein.

For example, polynucleotides are provided by this invention that comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, etc.; 21, 22, 23, etc.; 30, 31, 32, etc.; 50, 51, 52, 53, etc.; 100, 101, 102, 103, etc.; 150, 151, 152, 153, etc.; including all integers through 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention, for example polynucleotides that are optimized for human and/or primate codon selection. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

POLYNUCLEOTIDE IDENTIFICATION AND CHARACTERIZATION

Polynucleotides may be identified, prepared and/or manipulated using any of a variety of well established techniques. For example, a polynucleotide may be

identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (i.e., expression that is at least two fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena *et al.*, *Proc. Natl. Acad. Sci. USA* 93:10614-10619 (1996) and Heller *et al.*, *Proc. Natl. Acad. Sci. USA* 94:2150-2155 (1997)). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as *M. tuberculosis* cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (e.g., a *M. tuberculosis* cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (1989)). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques,

amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia *et al., Nucl. Acids Res.* 16:8186 (1988)), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom *et al., PCR Methods Applic.* 1:111-19 (1991)) and walking PCR (Parker *et al., Nucl. Acids. Res.* 19:3055-60 (1991)). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

POLYNUCLEOTIDE EXPRESSION IN HOST CELLS

In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or

functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (*see* Caruthers, M. H. *et al., Nucl. Acids Res. Symp. Ser.* pp. 215-223 (1980), Horn *et al., Nucl. Acids Res. Symp. Ser.* pp. 225-232 (1980)). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques

(Roberge *et al., Science* 269:202-204 (1995)) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, *Proteins, Structures and Molecular Principles* (1983)) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook *et al., Molecular Cloning, A Laboratory Manual* (1989), and Ausubel *et al., Current Protocols in Molecular Biology* (1989).

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example,

when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke &Schuster, *J. Biol. Chem.* 264:5503-5509 (1989)); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel *et al.* (*supra*) and Grant *et al.*, *Methods Enzymol.* 153:516-544 (1987).

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J.* 6:307-311 (1987)). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi *et al.*, *EMBO* J. *3*:1671-1680 (1984);

Broglie *et al., Science* 224:838-843 (1984); and Winter *et al., Results Probl. Cell Differ.* 17:85-105 (1991)). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*see, e.g.*, Hobbs *in McGraw Hill Yearbook of Science and Technology* pp. 191-196 (1992)).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia larvae*. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia larvae* in which the polypeptide of interest may be expressed (Engelhard *et al., Proc. Natl. Acad. Sci. U.S.A.* 91 :3224-3227 (1994)).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. U.S.A.* 81:3655-3659 (1984)). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of

expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf. *et al., Results Probl. Cell Differ.* 20:125-162 (1994)).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation. glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al., Cell* 11:223-32 (1977)) and adenine phosphoribosyltransferase (Lowy *et al., Cell* 22:817-23 (1990)) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler *et al., Proc. Natl. Acad. Sci. U.S.A.* 77:3567-70 (1980)); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al., J. Mol. Biol.* 150:1-14 (1981)); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan,

Proc. Natl. Acad. Sci. U.S.A. 85:8047-51 (1988)). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al., Methods Mol. Biol.* 55:121-131 (1995)).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton *et al., Serological Methods, a Laboratory Manual* (1990) and Maddox *et al., J. Exp. Med.* 158:1211-1216 (1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to

polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen. San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath *et al., Prot. Exp. Purif.* 3:263-281 (1992) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll *et al., DNA Cell Biol.* 12:441-453 (1993)).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963)). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

IN VIVO POLYNUCLEOTIDE DELIVERY TECHNIQUES

In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety or well known approaches, several of which are outlined below for the purpose of illustration.

1. *ADENOVIRUS*

One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus & Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is dispensable from the adenovirus genome (Jones & Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham & Prevec, 1991). In nature, adenovirus can package approximately 105% of the wild-type genome (Ghosh-Choudhury *et al.*, 1987), providing capacity for about 2 extra kB of DNA. Combined with the approximately 5.5 kB of DNA that is replaceable in the E1 and E3 regions, the maximum capacity of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-

borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, e.g., Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the currently preferred helper cell line is 293.

Recently, Racher *et al.* (1995) disclosed improved methods for culturing 293 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of

insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, e.g., 10^9-10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch *et al.*, 1963; Top *et al.*, 1971), demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors.

Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus & Horwitz, 1992; Graham & Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet & Perricaudet, 1991; Stratford-Perricaudet *et al.*, 1990; Rich *et al.*, 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.*, 1992), muscle injection (Ragot *et al.*, 1993), peripheral intravenous injections (Herz & Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993).

2. RETROVIRUSES

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5′ and 3′ ends of

the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding one or more oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas & Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

3. ADENO-ASSOCIATED VIRUSES

AAV (Ridgeway, 1988; Hermonat & Muzycska, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replications is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of

which AAV-2 is the best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka & McLaughlin, 1988).

The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs. There are two major genes in the AAV genome: *rep* and *cap*. The *rep* gene codes for proteins responsible for viral replications, whereas *cap* codes for capsid protein VP1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential *cis* components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of rep proteins, and transcription from p40 produces the capsid proteins (Hermonat & Muzyczka, 1984).

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering the antisense constructs of the present invention.

AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilize rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

4. Other Viral Vectors as Expression Constructs

Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar *et al.*, 1988), lentiviruses, polio viruses and herpes viruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar *et al.*, 1988; Horwich *et al.*, 1990).

With the recent recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich *et al.*, 1990). This suggested that large portions of the genome could be replaced with foreign genetic material. The hepatotropism and persistence (integration) were particularly attractive properties for liver-directed gene transfer. Chang *et al.* (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media containing high titers of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang *et al.*, 1991).

5. NON-VIRAL VECTORS

In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is *via* viral infection where the expression construct is encapsulated in an infectious viral particle.

Once the expression construct has been delivered into the cell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky *et al.* (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty & Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner *in vivo* and express the gene product.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded *in vivo* (Yang *et al.*, 1990; Zelenin *et al.*, 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, i.e., *ex vivo* treatment. Again, DNA encoding a particular gene may be delivered *via* this method and still be incorporated by the present invention.

POLYPEPTIDE COMPOSITIONS

The present invention, in other aspects, provides polypeptide compositions. Generally, a polypeptide of the invention will be an isolated polypeptide (or an epitope, variant, or active fragment thereof) derived from a mammalian species. Preferably, the polypeptide is encoded by a polynucleotide sequence disclosed herein or a sequence which hybridizes under moderately stringent conditions to a polynucleotide sequence disclosed herein. Alternatively, the polypeptide may be defined as a

polypeptide which comprises a contiguous amino acid sequence from an amino acid sequence disclosed herein, or which polypeptide comprises an entire amino acid sequence disclosed herein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a *Mycobacterium* sp. protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988). For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I-labeled Protein A.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally,

one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Polypeptides of the invention, immunogenic fragments thereof, and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc.* 85:2149-2146 (1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene* 40:39-46 (1985); Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258-8262 (1986); U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided. Such proteins comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see, e.g.*, Stoute *et al.*, *New Engl. J. Med.* 336:86-91 (1997)).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza* B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli*

(thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene* 43:265-292 (1986)). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology* 10:795-798 (1992)). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a *Mycobacterium* antigen. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; *see also* U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243).

Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a polypeptide of the invention, polynucleotide encoding such a polypeptide, and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, the polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a polypeptide of the invention if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* 54:1065-1070 (1994)). Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a polypeptide of the invention (100 ng/ml - 100 μg/ml, preferably 200 ng/ml - 25 μg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN-γ) is indicative of T cell activation (*see* Coligan *et al.*, *Current Protocols in Immunology*, vol. 1 (1998)). T cells that have been activated in response to a polypeptide, polynucleotide or polypeptide-expressing APC may be $CD4^+$ and/or $CD8^+$. Protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, $CD4^+$ or $CD8^+$ T cells that proliferate in response to a polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of

ways. For example, the T cells can be re-exposed to a polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize the polypeptide. Alternatively, one or more T cells that proliferate in the presence of the protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell and/or antibody compositions disclosed herein in pharmaceutically-acceptable or physiologically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. Such compositions are also useful for diagnostic uses.

It will also be understood that, if desired, the nucleic acid segment, RNA, DNA or PNA compositions that express a polypeptide as disclosed herein may be administered in combination with other agents as well, such as, e.g., other proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

1. ORAL DELIVERY

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be

enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz *et al.*, 1997; Hwang *et al.*, 1998; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. For

example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

2. INJECTABLE DELIVERY

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally as described in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U. S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged

absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion (*see, e.g., Remington's Pharmaceutical Sciences*, 15th Edition, pp. 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine,

histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

3. NASAL DELIVERY

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described e.g., in U. S. Patent 5,756,353 and U. S. Patent 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U. S. Patent 5,780,045 (specifically incorporated herein by reference in its entirety).

4. LIPOSOME-, NANOCAPSULE-, AND MICROPARTICLE-MEDIATED DELIVERY

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the nucleic acids or constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon & Papahadjopoulos, 1988; Allen and Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, 1990; Muller *et al.*, 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath & Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta & Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller & Baltimore, 1984), transcription factors and allosteric effectors (Nicolau & Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trails examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b; Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori & Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, i.e. in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977; 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered

by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for h or days, depending on their composition, and half lives in the blood range from min to several h. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific antigenic receptors located on a particular cell-

type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be are easily made, as described (Couvreur *et al.*, 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

VACCINES

In certain preferred embodiments of the present invention, vaccines are provided. The vaccines will generally comprise one or more pharmaceutical compositions, such as those discussed above, in combination with an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; *see, e.g.*, Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, Powell & Newman, eds., *Vaccine Design* (the subunit and adjuvant approach) (1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

Illustrative vaccines may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted

above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198 (1998), and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch *et al.*, *Proc. Natl. Acad. Sci. USA* 86:317-321 (1989); Flexner *et al.*, *Ann. N.Y. Acad. Sci.* 569:86-103 (1989); Flexner *et al.*, *Vaccine* 8:17-21 (1990); U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627 (1988); Rosenfeld *et al.*, *Science* 252:431-434 (1991); Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Kass-Eisler *et al.*, *Proc. Natl. Acad. Sci. USA* 90:11498-11502 (1993); Guzman *et al.*, *Circulation* 88:2838-2848 (1993); and Guzman *et al.*, *Cir. Res.* 73:1202-1207 (1993). Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science* 259:1745-1749 (1993) and reviewed by Cohen, *Science* 259:1691-1692 (1993). The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that a vaccine may comprise both a polynucleotide and a polypeptide component. Such vaccines may provide for an enhanced immune response.

It will be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (e.g., salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts).

While any suitable carrier known to those of ordinary skill in the art may be employed in the vaccine compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be

formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252. One may also employ a carrier comprising the particulate-protein complexes described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium* species or *Mycobacterium* derived proteins. For example, delipidated, deglycolipidated *M. vaccae* ("pVac") can be used. In another embodiment, BCG is used as an adjuvant. In addition, the vaccine can be administered to a subject previously exposed to BCG. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 and derivatives thereof (SmithKline Beecham, Philadelphia, PA); CWS, TDM, Leif, aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars;

cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (*e.g.*, IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.*, IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann & Coffman, *Ann. Rev. Immunol.* 7:145-173 (1989).

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; *see* US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science* 273:352 (1996). Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins . Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A, β-escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix,

particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamelar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol^{R} to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one preferred embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL® adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. Another particularly preferred adjuvant formulation employing QS21, 3D-MPL® adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 as disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2, AS2', AS2,'' SBAS-4, or SBAS6, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula (I): $HO(CH_2CH_2O)_n$-A-R,

wherein, n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, most preferably 9; the *R* component is C_{1-50}, preferably C_4-C_{20} alkyl

and most preferably C_{12} alkyl, and *A* is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12th edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes *et al.*, *Vaccine* 14:1429-1438 (1996)) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see, e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (i.e., matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau & Steinman, *Nature* 392:245-251 (1998)) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman & Levy, *Ann. Rev. Med.* 50:507-529 (1999)). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel *et al.*, *Nature Med.* 4:594-600 (1998)).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or

other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a protein (or portion or other variant thereof) such that the polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi *et al.*, *Immunology and Cell Biology* 75:456-460 (1997). Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or

aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

DIAGNOSTIC KITS

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a protein of the invention.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

EXAMPLES

The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

Example 1: Guinea pig vaccination with MTB72F fusion protein and compositions with individual antigens

Guinea pigs were immunized with adjuvant alone (SBAS1, SBAS2, or ASAS7 plus A1(OH)3), MTB72F fusion protein in adjuvant, or TbH9 plus Ra35 antigen composition.

Methods:

Groups:
1) SBAS1
2) SBAS2
3) SBAS7 + Al(OH)3
4) TbH9+Ra35 + SBAS1
5) TbH9 + Ra35 + SBAS2
6) TbH9 + Ra35 + SBAS7(Al(OH)3)
7) MTB72F in SBAS1
8) MTB72F in SBAS2
9) MTB72F in SBAS7+Al(OH)3
10) PBS
11) BCG

Dosage: 4 μg each of TbH9 and Ra35
8 μg MTB72F

Protocol: 1st immunization, 2nd immunization approximately 3 weeks later, 3rd immunization approximately two and a half weeks later.

Pre-challenge: DTH (delayed type hypersensitivity, used to determine antigenicity; 10 μg antigen)

Challenge: Aerosol with ~30 cfu Erdman strain

Post challenge monitoring: Weight loss

Death (~6 months post challenge)

Results:

1. DTH

Positive reaction to the immunizing antigens. Reactions to individual antigens or the fusion protein were comparable. Skin test reactivity to PPD was only seen with the BCG immunized groups

2. Protection: Guinea pigs vaccinated with MTB72F fusion protein afforded protection compared to those immunized with a mixture of antigens (*see* Figure 1).

Example 2: Mouse vaccination with MTB72F fusion protein and compositions with individual antigens

As described above, mice were immunized with adjuvant alone (SBAS2, SBAS2', SBAS2'', or SBAS6), MTB72F fusion protein in adjuvant, MTB72F DNA, MTB59F fusion protein in adjuvant, or TbH9, Ra35 and Ra12 antigen composition.

Methods:

Groups:

1) MTB72F+ SBAS2
2) MTB72F + SBAS2'
3) MTB72F + SBAS2''
4) MTB72F + SBAS6
5) Ra12+ TbH9 + Ra35 in SBAS2
6) MTB59F in SBAS2
7) SBAS2
8) MTB72F + delipidated, deglycolipidated *M. vaccae*
9) MTB72F DNA
10) MTB72F +IFA
11) MTB72F + BCG

12) delipidated, deglycolipidated *M. vaccae*

13) BCG

14) Saline

15) MTB72F +SBAS2 (in house formulation)

8 animals per group

Immunization schedule: First immunization, second immunization approximately 3 weeks later; third immunization approximately three weeks later.

Aerosol challenge approximately three months after first does

Spleen or lung cells were isolated and cultured; count CFU of cultures approximately three weeks after plating.

Dose: 8 μg MTB72F, 6.56 μg MTB59F, or 1.52, 4.3, and 2.24 μg, respectively, of Ra12, TbH9, and Ra35, mixed.

Results:

Of the AS adjuvants, AS2" + MTB72F gave the best protection in both the spleen and lung in this set of experiments (*see* Figures 2A and 2B). MTB72F gave ~1 log better protection than MTB59F in both spleen and lung in this set of experiments, indicating that Ra12 provides additional benefit. Mixture of 12/H9/35 + AS2 gave a better protection than MTB72F in this experiment. MTB72F DNA gave the best protection in this experiment, particularly in the spleen (>2 log). The protection was comparable in the lung to that seen with MTB72F protein + AS2", in this experiment.

Example 3: Guinea pig vaccination with MTB72F fusion protein and compositions with individual antigens

As described above, guinea pigs were immunized with adjuvant alone (SBAS2, SBAS2', SBAS2'', or SBAS6), MTB72F fusion protein in adjuvant, MTB72F DNA, MTB59F fusion protein in adjuvant, or TbH9, Ra35 and Ra12 antigen composition.

Methods:

Groups: 1) MTB72F + SBAS2

2) MTB72F + SBAS2'

3) MTB72F + SBAS2''
4) MTB72F + SBAS6
5) Ra12+ TbH9 + Ra35 in SBAS2
6) MTB59F in SBAS2
7) SBAS2
8) MTB72F + pvac
9) MTB72F DNA
10) MTB72F +IFA
11) MTB72F + BCG
12) BCG
13) Saline
14) delipidated, deglycolipidated *M. vaccae*

Antigens:

Antigens were formulated on a molar equivalent

5 animals per group

Injection volume per dose is 250µl (IM) containing

MTB72F	20 µg
Ra12, TbH9, Ra35	3.8, 10.8, and 5.6 µg
MTB59F	16.4 µg

Schedule:

1st immunization, 2nd immunization approximately three weeks later, 3rd immunization approximately three weeks later.

Challenge: ~ one and one half months after first immunization.

Results:

~38 Wks post challenge

Groups	Alive	State
G1. MTB72F + AS2	1/5	[losing weight]

G2. MTB72F + AS2'	2/5	[not gaining weight]
G3. MTB72F + AS2"	3/5	[looking okay, but no weight gain]
G4. MTB72F + AS6	2/5	[both these gaining weight]
G5. MTBRa12+H9+Ra35 +AS2	4/5	[one maybe a bit peaked, but two gaining]
G6. MTB59F + AS2	2/5	[both losing a little]
G7. AS2	2/5	[both losing]
G8. MTB72F + pVac	1/5	[not looking too good]
G9. MTB72F DNA	3/5	[all holding steady]
G10. MTB72F + IFA	2/5	[doing okay]
G11. MTB72F + BCG	5/5	[eating very well]
G12 BCG	4/5	[doing fine]
G13 Saline	all dead	
G14 pVac	2/5	[not gaining weight]

By 50 weeks post challenge, while 80% (4/5) of the guinea pigs immunized with BCG + Mtb72F were still alive, only 20% (1/5) of those immunized with BCG alone were alive. At 85 weeks, 4/5 of the guinea pigs immunized with BCG + Mtb72F were still alive and healthy (*see* Figure 7).

Example 4: Long term protection

As described above, guinea pigs were immunized with adjuvant alone (AS2 or AS2''), MTB72F fusion protein in adjuvant, TbH9, Ra35 and Ra12 antigen composition, or a variety of individual antigens in adjuvant.

Methods

GROUPS	ANTIGEN DOSE
1. AS2" + MTB39 (TbH9)	20ug/250ul (IM)
2. AS2" + MTB8.4 (DPV)	20ug
3. AS2" + MTB9.9 (MTI)	20ug
4. AS2" + MTB41 (MTCC#2)	20ug
5. AS2" + MTB40 (HTCC#1)	20ug
6. AS2" + MTB9.8 (MSL)	20ug
7. AS2" + MTB72F	20ug

8. AS2" + Ra12+TbH9 + Ra35 (molar equivalent) 3.8 μg +10.8 μg +5.6 μg

9. AS2" + MTB71F + MTB72F+HTCC#1 20 μg +20 μg +10 μg

10. AS2" + Ra12 20 μg

11. BCG

12. AS2"

13. AS2 + MTB72F

14. AS2+ Ra12+TbH9+Ra35

15. AS2

Example 5: Monkey vaccination with MTB72F fusion protein and compositions with individual antigens

As described above, monkeys were immunized with MTB72F fusion protein in SBAS2 adjuvant, or MTB8.4 antigen composition in adjuvant, or a mixture of MTB72F and MTB8.4.

Methods:

Groups

1. Saline
2. BCG
3. MTB8.4/AS2
4. MTB72F/AS2
5. MTB72F/AS2 (one arm) + MTB8.4/AS2 (other arm)

40 μg each antigen

Results:

At 8 weeks post challenge, monkeys immunized with BCG are showing signs of infection

Current data for 16 weeks post challenge reveals the following trend:

Groups immunized with MTB72F (4 and 5) are holding on their weights and have low ESR values compared to group 3 (MTB8.4 immunization) (Tables 1 and 2).

Table 1

Prophylactic Vaccine Study in Cynomolgus Monkeys with MTB8.4 and MTB72F formulated in AS2 20 Weeks Post Challenge

Groups	ID	Net weight Change (kg)	Chest X-ray (onset)	Status
	1398K	-24%	Pn, bil, prog (wk 8)	Alive
AS2	4437B	-33%	Pn, bil, prog (wk4)	Dead
	2959G	-8.30%	Pn, bil, prog (wk4)	Alive
	605AE	-14.00%	Pn, rt, stable (wk 8)	Alive
	3436A	-15.00%	Neg	Alive
BCG	3642G	Plus 4.5%	Pn, rt, prog (wk 8)	Alive
	1190H	0%	Neg	Alive
	1051I	-30%	Pn, rt, prog (wk 8)	Dead
	3665C	-25%	Pn, rt, prog (wk8)	Dead
MTB8.4	2200F	-18.00%	Pn, rt, stable (wk8)	Alive
	1654J	-33.00%	Pn, bil, prog (wk4)	Dead
	4141C	-33%	Pn, bil, prog (wk4)	Dead
	3061C*	Died after IT challenge		
MTB72F	1228G	Plus 3.6%	Bron, bil, stable for 3 mo (wk8)	Alive
	3462E	-2.20%	Neg	Alive
	4254C	Plus 1.21	Pn, rt, stable for 3 mo (wk4)	Alive
	4496A	Plus 7%	Pn, rt, stable for 1 mo (wk 8)	Alive
MTB8.4	4422C	-39.00%	Pn, bil, prog (wk 4)	Dead
MTB72F	4416A	Plus 11%	Pn, rt, stable for 2 mo (wk 12)	Alive
	2734E	Plus 12.5%	Susp infil rt, stable for 3 mo (wk 8)	Alive

Table 2

Prophylactic Vaccine Study in Cynomolgus Monkeys with MTB8.4 and MTB72F formulated in AS2

		Wks Post Challenge ESR				
Groups	ID	4	8	12	16	16 wks Chest X-ra
	1398K	3	3	10	19	Pn, bil, progrsv
AS2	4437B	10	20	3		Died
	2959G	6	3	3	0	Pn, rt, progrsv
	605AE	1	4	7	3	Pn, rt, stable
	3436A	0	8	7	15	Neg
BCG	3642G	0	0	0	0	Pn, rt, progrsv
	1190H	1	0	2	0	Neg
	1051I	0	8	22	7	Pn, bil, w/furt pro
						Died
	3665C	12	30	19		Died
MTB8.4	2200F	1	7	2	0	Pn, rt, progrsv
	1654J	20	8	21	7	Pn,bil,w/fur progr
	4141C	13	8	2	15	Pn,bil,w/fur progr
	3061C*	Died after IT challenge				
MTB72F	1228G	0	1	20	0	Now stable
	3462E	0	0	0	0	Neg
	4254C	13	0	0	0	Pn, now stable
	4496A	5	1	0	5	Pn, rt, w/furt prog
MTB8.4/	4422C	10	3	0		Died
MTB72F	4416A	6	0	1	0	Pn, now stable
	2734E	0	0	0	0	Susp infil, now st

Example 6: BCG priming experiment in monkeys

5 animals per group with four groups immunized with BCG and then rested, then immunized as described above and challenged. The following protocol will be used:

Groups	# animals	Immunizing Antigen	Antigen Dose
1. Nothing	5	AS2	
2. BCG	5	AS2	
3. BCG	5	MTB72F	40ug
4. BCG	4	Ra12+TbH9+Ra35	Molar equiv of antigens in MTB72F dose
5. BCG	4	MTB72F + MTB71F + MTB40	40ug MTB72F 40ug MTB72F 20ug MTB40

All antigens in formulated in AS2

Groups 4 and 5 have four animals each. Two of the BCG immunized monkeys died

Groups	# animals	Immunizing Antigen	Antigens for T cell proliferation and cytokine production assays
1. Nothing	5	AS2	PHA, PPD, MTB72F, MTB71F, HTCC#1, DPV, MTCC#2, Ra12, TbH9, Ra35, MSL, MTI
2. BCG	5	AS2	PHA, PPD, MTB72F, MTB71F, HTCC#1, DPV, MTCC#2, Ra12, TbH9, Ra35, MSL, MTI
3. BCG	5	MTB72F	PHA, PPD, MTB72F, Ra12, TbH9, Ra35
4. BCG	4	Ra12+TbH9+Ra35	PHA, PPD, MTB72F, Ra12, TbH9, Ra35
5. BCG	4	MTB72F + MTB71F + MTB40	PHA, PPD, MTB72F, MTB71F, HTCC#1, DPV, MTCC-2, Ra12, TbH9, Ra35, MSL, MTI

Example 7: Construction of Ra35MutSA and MTB72FMutSA

Expression of Mtb72f typically results in some breakdown products. In addition, the expression of the full-length sequences of the mature or full length form of Ra35 (Mtb32A) in *E. coli* has been difficult. The expressed product was only visible after immunoblotting with a polyclonal rabbit anti-Ra35 Ab indicative of low levels of protein expression. Even then, multiple specific species (bands) were detected indicative of auto-catalytic breakdown (degradation) of the recombinant antigen. This was presumed to be due to the expression of Ra35FL in *E. coli* as a biologically active form.

It has been previously shown that it was possible to express Ra35FL as two overlapping halves comprising the N-terminal (Ra35N-term, called Ra35) and C-term halves (Ra35C-term called Ra12). To enhance and stabilize the expression of the whole Ra35 molecule, a single point mutation was introduced at one of the residues

within the active-site triad (substitution of Ser to Ala; *see* Figures 6). This mutagenized form of Mtb32A can now be easily expressed at high levels in a stable form. In addition, to stabilize expression of Mtb72F, a single nucleotide substitution (T to G, resulting in a Ser to Ala change at position 710 of the fusion polypeptide) was incorporated in the sequence of Mtb72F at nucleotide position 2128 (*see* Figure 5).

This stabilization is also readily accomplished by mutagenizing any one, any two, or all three of the three residues comprising the active site triad in Ra35FL, Ra35, or Mtb72F or other fusion proteins comprising Ra35 (His, Asp, or Ser). Mutagenesis can be performed using any technique known to one of skill in the art.

Example 8: Immunization of mice withf Ra35FLMutSA-TbH9 and MTB72FMutSA

Eight mice per group were immunized with the compositions listed below, which include the adjuvant AS2A. The mice were then challenged with *Mycobacterium tuberculosis*, and survival of the mice was measured.

Group	Concentration of protein or DNA
1. Mtb72f protein	1.5 mg/ml
2. Mtb72f DNA	1.2 mg/ml
3. Mtb72f-85b protein	0.6 mg/ml
4. Mtb72f-85b DNA	1.1 mg/ml
5. Mtb72f-MTI protein	1.3 mg/ml
6. Mtb72f-MTI DNA	1.1 mg/ml
7. Mtb72f MutSA protein	1.7 mg/ml
8. MTB3AMutSA-TbH9 protein	2.4 mg/ml
9. BCG	
10. AS2	
11. vector alone	1.5 mg/ml
12. saline	

WHAT IS CLAIMED IS

1. A composition comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

2. The composition of claim 1, comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

3. The composition of claim 2, further comprising a polypeptide comprising at least about 132 amino acids from the C-terminus of MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

4. The composition of claims 1, 2, or 3, wherein the antigens are covalently linked, thereby forming a fusion polypeptide.

5. The composition of claim 4, wherein the fusion polypeptide has the amino acid sequence of MTB59F (SEQ ID NO:20).

6. The composition of claim 4, wherein the fusion polypeptide has the amino acid sequence of MTB72F (SEQ ID NO:16).

7. The composition of claim 4, wherein the fusion polypeptide has the amino acid sequence of MTB72FMutSA (SEQ ID NO:18).

8. The composition of claim 6 or 7, further comprising BCG.

9. The composition of claim 6 or 7, further comprising at least one additional antigen from a *Mycobacterium* species of the tuberculosis complex, wherein the antigen is selected from the group consisting of MTB8.4 antigen (SEQ ID NO:22), MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen (SEQ ID NO:27), MTB40 antigen (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31), 38-1 (SEQ ID NO:35), TbRa3 (SEQ ID NO:37), 38 kD (SEQ ID NO:39), DPEP (SEQ ID NO:41), TbH4 (SEQ ID NO:43),

DPPD(SEQ ID NO:45), MTB82, Erd14, ESAT-6 antigen (SEQ ID NO:33), MTB85 complex antigen, or α-crystalline antigen, or an immunogenic fragment thereof.

10. The composition of claim 6 or 7, further comprising an adjuvant.

11. The composition of claim 4, wherein the antigens are covalently linked via a chemical linker.

12. The composition of claim 11, wherein the chemical linker is an amino acid linker.

13. The composition of claim 1, further comprising at least one additional antigen from a *Mycobacterium* species of the tuberculosis complex, wherein the antigen is selected from the group consisting ofMTB8.4 antigen (SEQ ID NO:22), MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen (SEQ ID NO:27), MTB40 antigen (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31), 38-1 (SEQ ID NO:35), TbRa3 (SEQ ID NO:37), 38 kD (SEQ ID NO:39), DPEP (SEQ ID NO:41), TbH4 (SEQ ID NO:43), DPPD(SEQ ID NO:45), MTB82, Erd14, ESAT-6 antigen (SEQ ID NO:33), MTB85 complex antigen, or α-crystalline antigen, or an immunogenic fragment thereof.

14. The composition of claim 1, further comprising an adjuvant.

15. The composition of claim 14, wherein the adjuvant comprises QS21 and MPL.

16. The composition of claim 14, wherein the adjuvant is selected from the group consisting of AS2, ENHANZYN, MPL, 3D-MPL, IFA, QS21, CWS, TDM, AGP, CPG, Leif, saponin, and saponin mimetics.

17. The composition of claim 1, further comprising BCG or pVac.

18. The composition of claim 1, further comprising an NS1 antigen or an immunogenic fragment thereof.

19. The composition of claim 1, wherein the *Mycobacterium* species is *Mycobacterium tuberculosis*.

20. An expression cassette comprising a nucleic acid encoding a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a nucleic acid encoding a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

21. The expression cassette of claim 20, comprising a nucleic acid encoding a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a nucleic acid encoding a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO: 2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

22. The expression cassette of claim 21, further comprising a nucleic acid encoding a polypeptide comprising at least 132 amino acids of the C-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

23. The expression cassette of claim 20, wherein the nucleic acid encodes a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof and a nucleic acid encoding a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic fragment thereof.

24. The expression cassette of claim 23, wherein the nucleic acid encodes a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof, and a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4).

25. The expression cassette of claim 24, wherein the fusion polypeptide further comprises a polypeptide comprising at least 132 amino acids of the C-terminus of a MTB32A antigen (SEQ ID NO:2 or 4).

26. The expression cassette of claim 24, wherein the nucleic acid encodes a fusion polypeptide having the amino acid sequence of MTB59F (SEQ ID NO:20).

27. The expression cassette of claim 26, wherein the nucleic acid has the sequence of the nucleic acid encoding MTB59F (SEQ ID NO:19).

28. The expression cassette of claim 25, wherein the nucleic acid encodes a fusion polypeptide having the amino acid sequence of MTB72F (SEQ ID NO:16).

29. The expression cassette of claim 28, wherein the nucleic acid has the sequence of the nucleic acid encoding MTB72F (SEQ ID NO:15).

30. The expression cassette of claim 28, wherein the nucleic acid has the sequence of the nucleic acid encoding MTB72FMutSA (SEQ ID NO:18).

31. The expression cassette of claim 29or 30, further comprising a nucleic acid encoding at least one additional antigen from a *Mycobacterium* species of the tuberculosis complex, wherein the antigen is selected from the group consisting ofMTB8.4 antigen (SEQ ID NO:22), MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen (SEQ ID NO:27), MTB40 antigen (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31), 38-1 (SEQ ID NO:35), TbRa3 (SEQ ID NO:37), 38 kD (SEQ ID NO:39), DPEP (SEQ ID NO:41), TbH4 (SEQ ID NO:43), DPPD(SEQ ID NO:45), MTB82, Erd14, ESAT-6 antigen (SEQ ID NO:33), MTB85 complex antigen, or α-crystalline antigen, or an immunogenic fragment thereof.

32. The expression cassette of claim 20, further comprising a nucleic acid encoding at least one additional antigen from a *Mycobacterium* species of the tuberculosis complex, wherein the antigen is selected from the group consisting ofMTB8.4 antigen (SEQ ID NO:22), MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen (SEQ ID NO:27), MTB40 antigen (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31), 38-1 (SEQ ID NO:35), TbRa3 (SEQ ID NO:37), 38 kD (SEQ ID NO:39), DPEP (SEQ ID NO:41), TbH4 (SEQ ID NO:43), DPPD(SEQ ID NO:45), MTB82, Erd14, ESAT-6 antigen (SEQ ID NO:33), MTB85 complex antigen, or α-crystalline antigen, or an immunogenic fragment thereof.

33. The expression cassette of claim 20, further comprising a nucleic acid encoding an NS1 antigen.

34. The expression cassette of claim 20, wherein the *Mycobacterium* species is *Mycobacterium tuberculosis*.

35. A method for eliciting an immune response in a mammal, the method comprising the step of administering to the mammal an immunologically effective amount of a pharmaceutical composition comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

36. The method of claim 35, wherein the mammal has been immunized with BCG.

37. The method of claim 35, wherein the mammal is a human.

38. The method of claim 35, wherein the composition is administered prophylactically.

39. The method of claim 35, comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

40. The method of claim 39, further comprising a polypeptide comprising at least about 132 amino acids from the C-terminus of MTB32A antigen (SEQ ID NO: 2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

41. The method of claim 35 or 39, wherein the antigens are covalently linked, thereby forming a fusion protein.

42. The method of claim 41, wherein the fusion polypeptide has the amino acid sequence of MTB59F (SEQ ID NO:20).

43. The method of claim 40, wherein the antigens are covalently linked, thereby forming a fusion protein.

44. The method of claim 43, wherein the fusion polypeptide has the amino acid sequence of MTB72F (SEQ ID NO:16).

45. The method of claim 43, wherein the fusion polypeptide has the amino acid sequence of MTB72FMutSA (SEQ ID NO:18).

46. The method of claim 35, wherein the pharmaceutical composition further comprises an adjuvant.

47. The method of claim 46, wherein the adjuvant comprises QS21 and MPL.

48. The method of claim 46, wherein the adjuvant is selected from the group consisting of AS2, ENHANZYN, MPL, 3D-MPL, IFA, QS21, CWS, TDM, AGP, CPG, Leif, saponin, and saponin mimetics.

49. A method for eliciting an immune response in a mammal, the method comprising the step of administering to the mammal an immunologically effective amount of an expression cassette comprising a nucleic acid encoding a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a nucleic acid encoding a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

50. The method of claim 49, wherein the mammal has been immunized with BCG.

51. The method of claim 49, wherein the mammal is a human.

52. The method of claim 49, wherein the composition is administered prophylactically.

53. The method of claim 49, wherein the nucleic acid encodes a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof, and a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) .

54. The method of claim 53, further comprising a nucleic acid encoding a polypeptide comprising at least 132 amino acids of the C-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

55. The method of claim 49, wherein the nucleic acid encodes a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO: 12 or 14) or an immunogenic fragment thereof and a nucleic acid encoding a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic fragment thereof.

56. The method of claim 55, wherein the nucleic acid encodes a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof, and a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO: 2 or 4).

57. The method of claim 56, wherein the fusion polypeptide further comprises a polypeptide comprising at least 132 amino acids of the C-terminus of a MTB32A antigen (SEQ ID NO:2 or 4).

58. The method of claim 56, wherein the nucleic acid encodes a fusion polypeptide having the amino acid sequence of MTB59F (SEQ ID NO:20).

59. The method of claim 58, wherein the nucleic acid has the nucleotide sequence of the nucleic acid encoding MTB59F (SEQ IDNO:19).

60. The method of claim 57, wherein the nucleic acid encodes a fusion polypeptide having the amino acid sequence of MTB72F (SEQ ID NO:16) .

61. The method of claim 57, wherein the nucleic acid encodes a fusion polypeptide having the amino acid sequence of MTB72FMutSA (SEQ ID NO:18).

62. The method of claim 60, wherein the nucleic acid has the nucleotide sequence of the nucleic acid encoding MTB72F (SEQ IDNO:15).

63. The method of claim 60, wherein the nucleic acid has the nucleotide sequence of the nucleic acid encoding MTB72FMutSA (SEQ ID NO:17).

64. An isolated nucleic acid encoding a MTB32A antigen from a *Mycobacterium* species of the tuberculosis complex, wherein at least one amino acid in the active site triad of the MTB32A antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

65. The nucleic acid of claim 64, wherein an serine residue corresponding to amino acid position 183 of SEQ ID NO:4 or position 207 of SEQ ID NO:2 has been substituted by another amino acid.

66. The nucleic acid of claim 65, wherein an alanine residue has been substituted for the serine residue.

67. The nucleic acid of claim 66, wherein the nucleic acid comprises a nucleotide sequence of SEQ ID NO:5.

68. A composition comprising the nucleic acid of claim 64.

69. A nucleic acid encoding a fusion polypeptide comprising the nucleic acid of claim 64.

70. An isolated MTB32A polypeptide from a *Mycobacterium* species of the tuberculosis complex, wherein at least one amino acid in the active site triad of the MTB32A antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

71. The polypeptide of claim 70, wherein a serine residue corresponding to amino acid position 183 of SEQ ID NO:4 or amino acid position 207 of SEQ ID NO:2 has been substituted by another amino acid.

72. The polypeptide of claim 71, wherein an alanine residue has been substituted for the serine residue.

73. A polypeptide of claim 72, wherein the polypeptide comprises an amino acid sequence of SEQ ID NO:6.

74. A composition comprising the polypeptide of claim 70.

75. A fusion polypeptide comprising the polypeptide of claim 70.

76. An isolated nucleic acid encoding a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) from a *Mycobacterium* species of the tuberculosis complex, and an antigen comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex, wherein an amino acid of the active site triad of the MTB32A antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

77. The nucleic acid of claim 76, wherein a serine residue corresponding to amino acid at position 183 of SEQ ID NO:4 or position 207 or SEQ ID NO:2 has been substituted by another amino acid.

78. The nucleic acid of claim 77, wherein an alanine residue has been substituted for the serine residue.

79. A composition comprising the nucleic acid of claim 76.

80. A nucleic acid encoding a fusion polypeptide comprising the nucleic acid of claim 76.

81. A nucleic acid encoding a fusion polypeptide, wherein the nucleic acid comprises a nucleotide sequence of SEQ ID NO:17.

82. A nucleic acid encoding a fusion polypeptide comprising an amino acid sequence of SEQ ID NO:18.

83. An isolated polypeptide encoding a fusion polypeptide comprising a MTB39 (SEQ ID NO: 12 or 14) antigen from a *Mycobacterium* species of the tuberculosis complex, and an antigen comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex, wherein an amino acid of the active site triad of the MTB32A antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

84. The polypeptide of claim 83, wherein an serine residue corresponding to amino acid position 183 of SEQ ID NO:4 or amino acid position 207 of SEQ ID NO:2 has been substituted by another amino acid.

85. The polypeptide of claim 83, wherein an alanine residue has been substituted for the serine residue.

86. A composition comprising the polypeptide of claim 83.

87. A fusion polypeptide comprising the polypeptide of claim 83.

88. A fusion polypeptide comprising an amino acid sequence of SEQ ID NO:18.

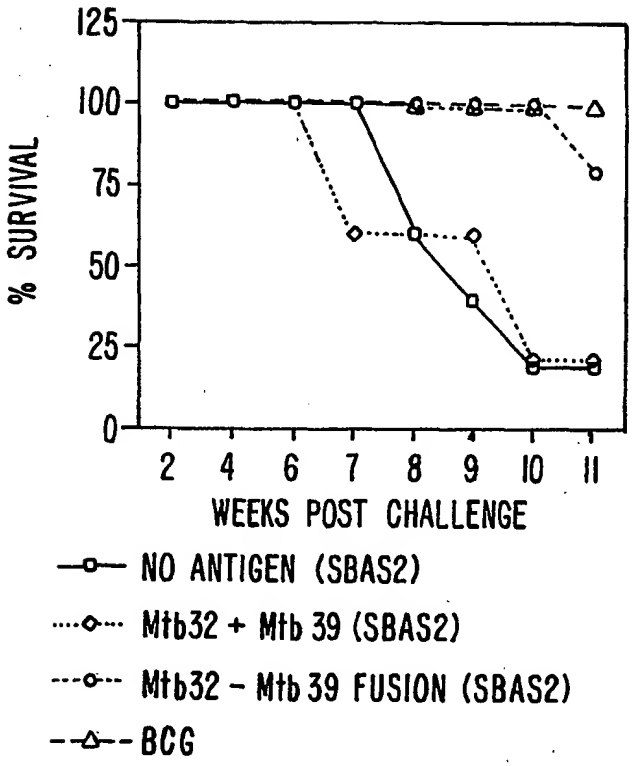
125
100
75
50
25
0
% SURVIVAL
2
4
6
7
8
9
10
11
WEEKS POST CHALLENGE
NO ANTIGEN (SBAS2)
Mtb32 + Mtb 39 (SBAS2)
Mtb32 - Mtb 39 FUSION (SBAS2)
BCG

FIG. 1.

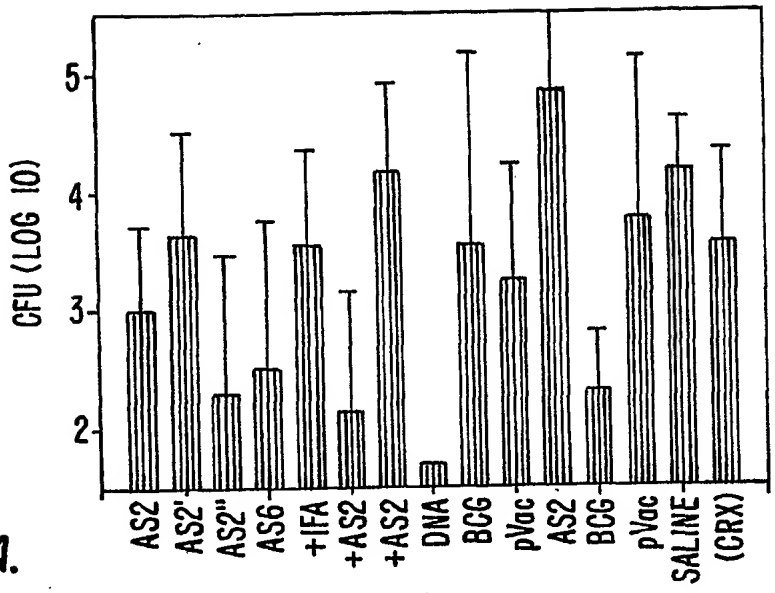
CFU (LOG 10)
5
4
3
2
AS2
AS2'
AS2"
AS6
+IFA
+AS2
+AS2
DNA
BCG
pVac
AS2
BCG
pVac
SALINE
(CRX)

FIG. 2A.

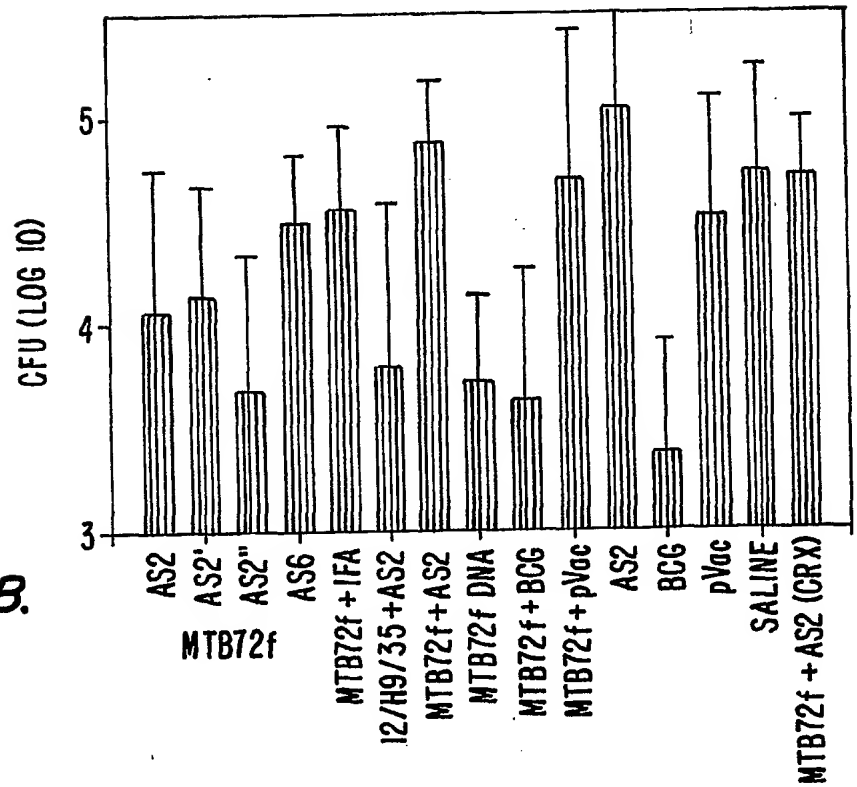
CFU (LOG 10)
5
4
3
AS2
AS2'
AS2"
AS6
MTB72f + IFA
12/H9/35 + AS2
MTB72f + AS2
MTB72f DNA
MTB72f + BCG
MTB72f + pVac
AS2
BCG
pVac
SALINE
MTB72f + AS2 (CRX)
MTB72f

FIG. 2B.

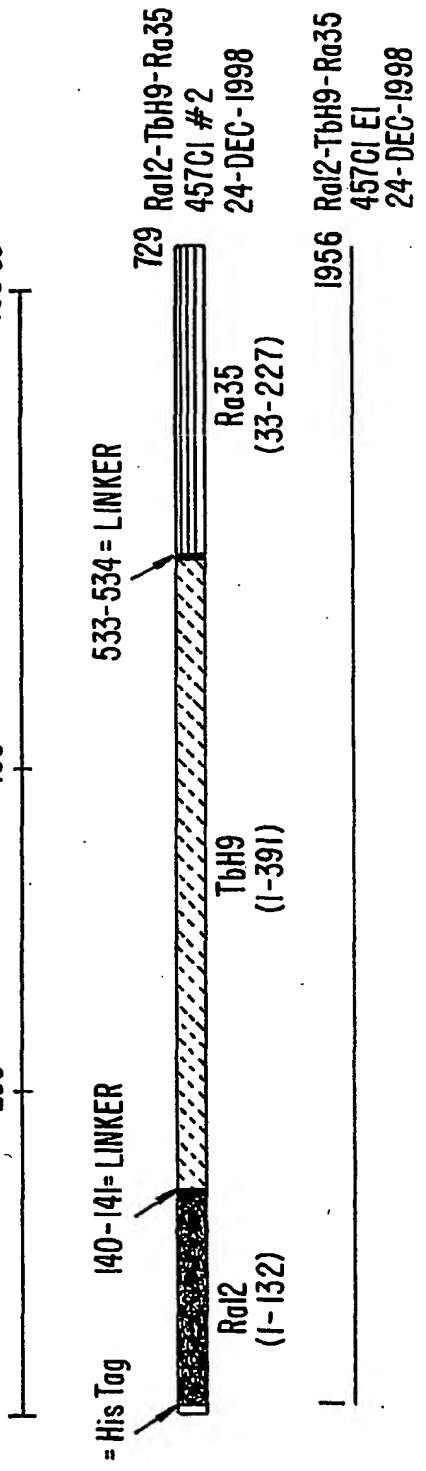
700 aa
400
200
729
1956
1
Ral2-TbH9-Ra35
457CI #2
24-DEC-1998
Ral2-TbH9-Ra35
457CI E1
24-DEC-1998
533-534 = LINKER
140-141= LINKER
= His Tag
Ra35
(33-227)
TbH9
(1-391)
Ral2
(1-132)

FIG. 3.

Ra35 N-terminus DNA

```
gccccgccgg ccttgtcgca ggaccggttc gccgacttcc ccgcgctgcc cctcgacccg tccgcgatgg 70
tcgcccaagt ggggccacag gtggtcaaca tcaacaccaa actgggctac aacaacgccg tgggcgccgg 140
gaccggcatc gtcatcgatc ccaacggtgt cgtgctgacc aacaaccacg tgatcgcggg cgccaccgac 210
atcaatgcgt tcagcgtcgg ctccggccaa acctacggcg tcgatgtggt cgggtatgac cgcacccagg 280
atgtcgcggt gctgcagctg cgcggtgccg gtggcctacc atcggcggcg atcggtggcg gcgtcgcggt 350
tggtgagccc gtcgtcgcga tgggcaacag cggtgggcag ggcggaacgc cccgtgcggt gcctggcagg 420
gtggtcgcgc tcggccaaac cgtgcaggcg tcggattcgc tgaccggtgc cgaagagaca ttgaacgggt 490
tgatccagtt cgatgccgcg atccagcccg gtgattcggg cgggcccgtc gtcaacggcc taggacaggt 560
ggtcggtatg aacacggccg cgtcctag                                          588
```

Ra35 N-terminus amino acid sequence

```
Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala
                5                  10                  15                  20

Met Val Ala Gln Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val
            25                  30                  35                  40

Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val Ile Ala
        45                  50                  55                  60                  65

Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly
                70                  75                  80                  85

Tyr Asp Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala
            90                  95                  100                 105                 110
```

FIG. 4.

```
Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly Gly Gln Gly Gly
                115                 120                 125                 130

Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu
            135                 140                 145                 150

Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
155                 160                 165                 170                 175

Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr Ala Ala Ser
                180                 185                 190                 195
```

FIG. 4. (CONTINUED)

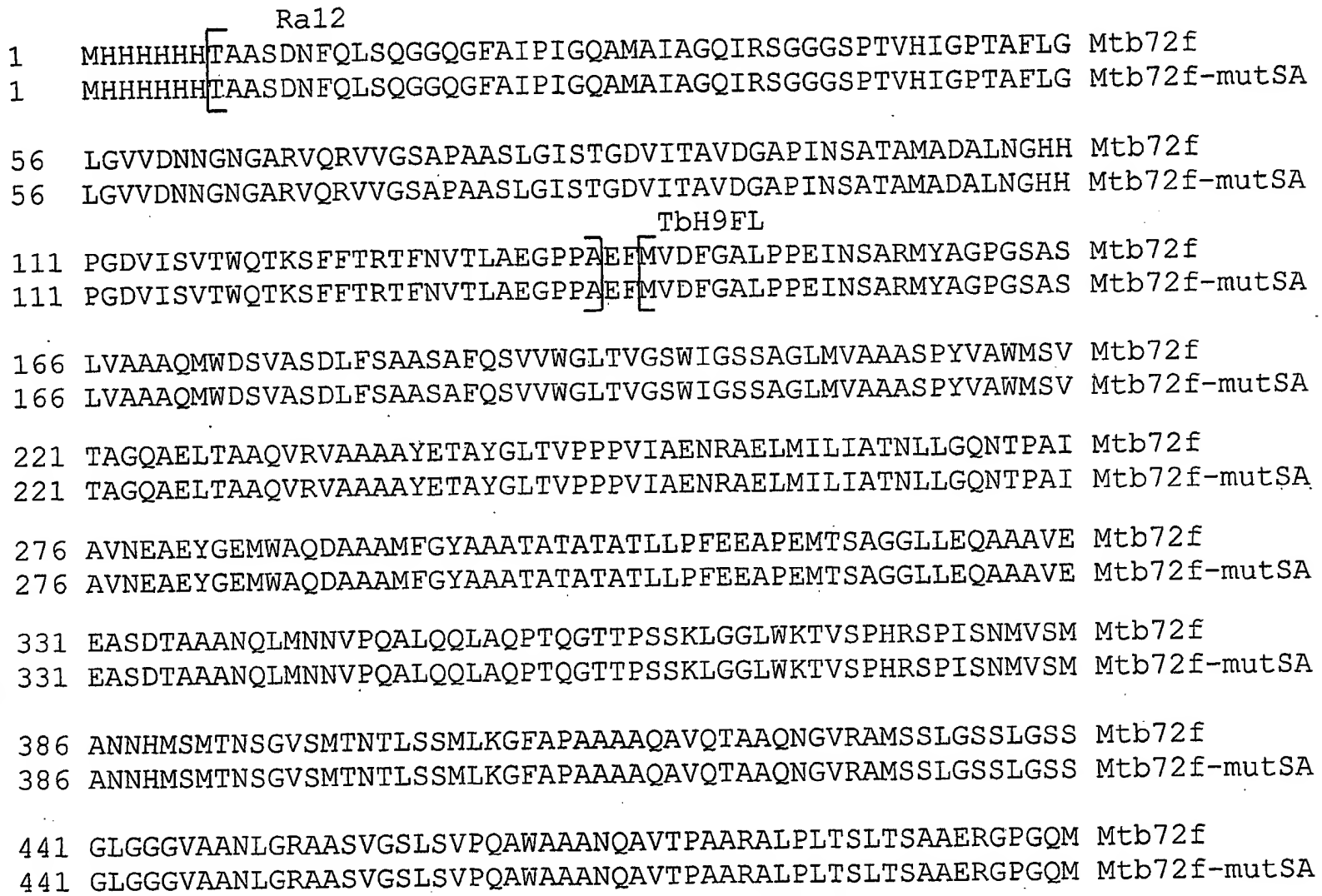

FIG. 5.

```
                                        Ra35
496 LGGLPVGQMGARAGGGLSGVLRVPPRPYVMPHSPAAGDIAPPALSQDRFADFPAL Mtb72f
496 LGGLPVGQMGARAGGGLSGVLRVPPRPYVMPHSPAAGDIAPPALSQDRFADFPAL Mtb72f-mutSA

551 PLDPSAMVAQVGPQVVNINTKLGYNNAVGAGTGIVIDPNGVVLTNNNVIAGATDI Mtb72f
551 PLDPSAMVAQVGPQVVNINTKLGYNNAVGAGTGIVIDPNGVVLTNNHVIAGATDI Mtb72f-mutSA

606 NAFSVGSGQTYGVDVVGYDRTQDVAVLQLRGAGGLPSAAIGGGVAVGEPVVAMGN Mtb72f
606 NAFSVGSGQTYGVDVVGYDRTQDVAVLQLRGAGGLPSAAIGGGVAVGEPVVAMGN Mtb72f-mutSA

661 SGGQGGTPRAVPGRVVALGQTVQASDSLTGAEETLNGLIQFDAAIQPGDSGGPVV Mtb72f
661 SGGQGGTPRAVPGRVVALGQTVQASDSLTGAEETLNGLIQFDAAIQPGDAGGPVV Mtb72f-mutSA

716 NGLGQVVGMNTAAS                                          Mtb72f
716 NGLGQVVGMNTAAS                                          Mtb72f-mutSA
```

FIG. 5. (CONTINUED)

```
                Ra35 N-term
1    MHHHHHHAPPALSQDRFADFPALPLDPSAMVAQVGPQVVNINTKLGYNNA TbRa35_mat
1    MHHHHHHAPPALSQDRFADFPALPLDPSAMVAQVGPQVVNINTKLGYNNA TbRa35 mutSA

51   VGAGTGIVIDPNGVVLTNNHVIAGATDINAFSVGSGQTYGVDVVGYDRTQ TbRa35_mat
51   VGAGTGIVIDPNGVVLTNNHVIAGATDINAFSVGSGQTYGVDVVGYDRTQ TbRa35 mutSA

101  DVAVLQLRGAGGLPSAAIGGGVAVGEPVVAMGNSGGQGGTPRAVPGRVVA TbRa35_mat
101  DVAVLQLRGAGGLPSAAIGGGVAVGEPVVAMGNSGGQGGTPRAVPGRVVA TbRa35 mutSA
                                                  Ra12 Cterm
151  LGQTVQASDSLTGAEETLNGLIQFDAAIQPGDSGGPVVNGLGQVVGMNTA TbRa35_mat
151  LGQTVQASDSLTGAEETLNGLIQFDAAIQPGDAGGPVVNGLGQVVGMNTA TbRa35 mutSA
       end Ra35 Nterm
201  ASDNFQLSQGGQGFAIPIGQAMAIAGQIRSGGGSPTVHIGPTAFLGLGVV TbRa35_mat
201  ASDNFQLSQGGQGFAIPIGQAMAIAGQIRSGGGSPTVHIGPTAFLGLGVV TbRa35 mutSA

251  DNNGNGARVQRVVGSAPAASLGISTGDVITAVDGAPINSATAMADALNGH TbRa35_mat
251  DNNGNGARVQRVVGSAPAASLGISTGDVITAVDGAPINSATAMADALNGH TbRa35 mutSA

301  HPGDVISVTWQTKSGGTRTGNVTLAEGPPA  end                 TbRa35_mat
301  HPGDVISVTWQTKSGGTRTGNVTLAEGPPA  Ra12                TbRa35 mutSA
```

FIG. 6.

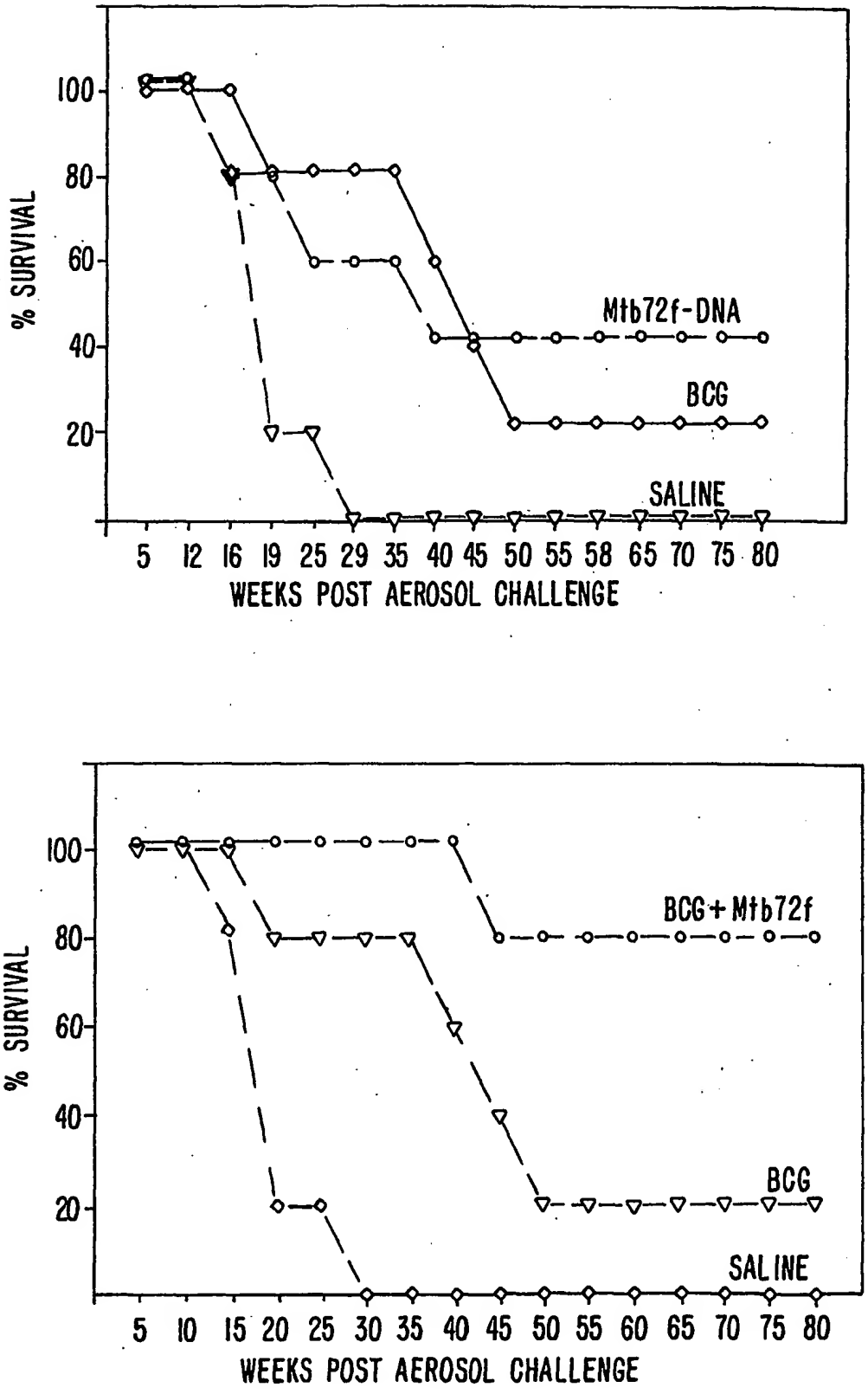
% SURVIVAL
100
80
60
40
20
Mtb72f-DNA
BCG
SALINE
5 12 16 19 25 29 35 40 45 50 55 58 65 70 75 80
WEEKS POST AEROSOL CHALLENGE
% SURVIVAL
100
80
60
40
20
BCG+Mtb72f
BCG
SALINE
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
WEEKS POST AEROSOL CHALLENGE

FIG. 7.

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:1: MTB32A (Ra35 FL)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1872 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACTACGTTG GTGTAGAAAA ATCCTGCCGC CCGGACCCTT AAGGCTGGGA CAATTTCTGA 60
TAGCTACCCC GACACAGGAG GTTACGGGAT GAGCAATTCG CGCCGCCGCT CACTCAGGTG 120
GTCATGGTTG CTGAGCGTGC TGGCTGCCGT CGGGCTGGGC CTGGCCACGG CGCCGGCCCA 180
GGCGGCCCCG CCGGCCTTGT CGCAGGACCG GTTCGCCGAC TTCCCCGCGC TGCCCCTCGA 240
CCCGTCCGCG ATGGTCGCCC AAGTGGCGCC ACAGGTGGTC AACATCAACA CCAAACTGGG 300
CTACAACAAC GCCGTGGGCG CCGGGACCGG CATCGTCATC GATCCCAACG GTGTCGTGCT 360
GACCAACAAC CACGTGATCG CGGGCGCCAC CGACATCAAT GCGTTCAGCG TCGGCTCCGG 420
CCAAACCTAC GGCGTCGATG TGGTCGGGTA TGACCGCACC CAGGATGTCG CGGTGCTGCA 480
GCTGCGCGGT GCCGGTGGCC TGCCGTCGGC GGCGATCGGT GGCGGCGTCG CGGTTGGTGA 540
GCCCGTCGTC GCGATGGGCA ACAGCGGTGG GCAGGGCGGA ACGCCCCGTG CGGTGCCTGG 600
CAGGGTGGTC GCGCTCGGCC AAACCGTGCA GGCGTCGGAT TCGCTGACCG GTGCCGAAGA 660
GACATTGAAC GGGTTGATCC AGTTCGATGC CGCAATCCAG CCCGGTGATT CGGGCGGGCC 720
CGTCGTCAAC GGCCTAGGAC AGGTGGTCGG TATGAACACG GCCGCGTCCG ATAACTTCCA 780
GCTGTCCCAG GGTGGGCAGG GATTCGCCAT TCCGATCGGG CAGGCGATGG CGATCGCGGG 840
CCAAATCCGA TCGGGTGGGG GGTCACCCAC CGTTCATATC GGGCCTACCG CCTTCCTCGG 900
CTTGGGTGTT GTCGACAACA ACGGCAACGG CGCACGAGTC CAACGCGTGG TCGGAAGCGC 960
TCCGGCGGCA AGTCTCGGCA TCTCCACCGG CGACGTGATC ACCGCGGTCG ACGGCGCTCC 1020
GATCAACTCG GCCACCGCGA TGGCGGACGC GCTTAACGGG CATCATCCCG GTGACGTCAT 1080
CTCGGTGAAC TGGCAAACCA AGTCGGGCGG CACGCGTACA GGGAACGTGA CATTGGCCGA 1140
GGGACCCCCG GCCTGATTTG TCGCGGATAC CACCCGCCGG CCGGCCAATT GGATTGGCGC 1200
CAGCCGTGAT TGCCGCGTGA GCCCCCGAGT TCCGTCTCCC GTGCGCGTGG CATTGTGGAA 1260
GCAATGAACG AGGCAGAACA CAGCGTTGAG CACCCTCCCG TGCAGGGCAG TTACGTCGAA 1320
GGCGGTGTGG TCGAGCATCC GGATGCCAAG GACTTCGGCA GCGCCGCCGC CCTGCCCGCC 1380
GATCCGACCT GGTTTAAGCA CGCCGTCTTC TACGAGGTGC TGGTCCGGGC GTTCTTCGAC 1440
GCCAGCGCGG ACGGTTCCGN CGATCTGCGT GGACTCATCG ATCGCCTCGA CTACCTGCAG 1500
TGGCTTGGCA TCGACTGCAT CTGTTGCCGC CGTTCCTACG ACTCACCGCT GCGCGACGGC 1560
GGTTACGACA TTCGCGACTT CTACAAGGTG CTGCCCGAAT TCGGCACCGT CGACGATTTC 1620
GTCGCCCTGG TCGACACCGC TCACCGGCGA GGTATCCGCA TCATCACCGA CCTGGTGATG 1680
AATCACACCT CGGAGTCGCA CCCCTGGTTT CAGGAGTCCC GCCGCGACCC AGACGGACCG 1740
TACGGTGACT ATTACGTGTG GAGCGACACC AGCGAGCGCT ACACCGACGC CCGGATCATC 1800
TTCGTCGACA CCGAAGAGTC GAACTGGTCA TTCGATCCTG TCCGCCGACA GTTNCTACTG 1860
GCACCGATTC TT 1872

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 355 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: MTB32A (Ra35FL)

Met Ser Asn Ser Arg Arg Arg Ser Leu Arg Trp Ser Trp Leu Leu Ser
1 5 10 15
Val Leu Ala Ala Val Gly Leu Gly Leu Ala Thr Ala Pro Ala Gln Ala
20 25 30
Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu
35 40 45
Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Ala Pro Gln Val Val
50 55 60
Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr
65 70 75 80

```
Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val
                85                  90                  95
Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln
            100                 105                 110
Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala
        115                 120                 125
Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly
    130                 135                 140
Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly
145                 150                 155                 160
Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu
                165                 170                 175
Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr
            180                 185                 190
Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
        195                 200                 205
Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr
    210                 215                 220
Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe Ala
225                 230                 235                 240
Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser Gly
                245                 250                 255
Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu
            260                 265                 270
Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val
        275                 280                 285
Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile
    290                 295                 300
Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp
305                 310                 315                 320
Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Asn Trp Gln
                325                 330                 335
Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly
            340                 345                 350
Pro Pro Ala
        355
```

<212> DNA
<213> Ra35 mature
<400> SEQ ID NO:3

```
catatgcatc accatcacca tcacgccccg ccggccttgt cgcaggaccg gttcgccgac 60
ttccccgcgc tgcccctcga cccgtccgcg atggtcgccc aagtggggcc acaggtggtc 120
aacatcaaca ccaaactggg ctacaacaac gccgtgggcg ccgggaccgg catcgtcatc 180
gatcccaacg gtgtcgtgct gaccaacaac cacgtgatcg cgggcgccac cgacatcaat 240
gcgttcagcg tcggctccgg ccaaacctac ggcgtcgatg tggtcgggta tgaccgcacc 300
caggatgtcg cggtgctgca gctgcgcggt gccggtggcc tgccgtcggc ggcgatcggt 360
ggcggcgtcg cggttggtga gcccgtcgtc gcgatgggca acagcggtgg gcagggcgga 420
acgccccgtg cggtgcctgg cagggtggtc gcgctcggcc aaaccgtgca ggcgtcggat 480
tcgctgaccg gtgccgaaga gacattgaac gggttgatcc agttcgatgc cgcgatccag 540
cccggtgagg cgggcgggcc cgtcgtcaac ggcctaggac aggtggtcgg tatgaacacg 600
gccgcgtccg ataacttcca gctgtcccag ggtgggcagg gattcgccat tccgatcggg 660
caggcgatgg cgatcgcggg ccagatccga tcgggtgggg ggtcacccac cgttcatatc 720
gggcctaccg ccttcctcgg cttgggtgtt gtcgacaaca acggcaacgg cgcacgagtc 780
caacgcgtgg tcgggagcgc tccggcggca agtctcggca tctccaccgg cgacgtgatc 840
accgcggtcg acggcgctcc gatcaactcg gccaccgcga tggcggacgc gcttaacggg 900
catcatcccg gtgacgtcat ctcggtgacc tggcaaacca agtcgggcgg cacgcgtaca 960
gggaacgtga cattggccga gggacccccg gcctgagaat tc                    1002
```

<212> PRT
<213> Ra35 mature
<400> SEQ ID NO:4

```
Met His His His His His His Ala Pro Pro Ala Leu Ser Gln Asp Arg
                5                   10                  15
```

Phe Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala
20 25 30

Gln Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn
35 40 45

Asn Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val
50 55 60

Val Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala
65 70 75 80

Phe Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr
85 90 95

Asp Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly
100 105 110

Leu Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val
115 120 125

Val Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val
130 135 140

Pro Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser
145 150 155 160

Leu Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala
165 170 175

Ala Ile Gln Pro Gly Asp Ser Gly Gly Pro Val Val Asn Gly Leu Gly
180 185 190

Gln Val Val Gly Met Asn Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser
195 200 205

Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile
210 215 220

Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly
225 230 235 240

Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly
245 250 255

Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly
260 265 270

Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn
275 280 285

Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp
290 295 300

Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly
305 310 315 320

Asn Val Thr Leu Ala Glu Gly Pro Pro Ala
325 330

<212> DNA
<213> Ra35FLMutSA
<400> SEQ ID NO:5

catatgcatc accatcacca tcacgccccg ccggccttgt cgcaggaccg gttcgccgac 60
ttccccgcgc tgcccctcga cccgtccgcg atggtcgccc aagtggggcc acaggtggtc 120
aacatcaaca ccaaactggg ctacaacaac gccgtgggcg ccgggaccgg catcgtcatc 180

```
gatcccaacg gtgtcgtgct gaccaacaac cacgtgatcg cgggcgccac cgacatcaat 240
gcgttcagcg tcggctccgg ccaaacctac ggcgtcgatg tggtcgggta tgaccgcacc 300
caggatgtcg cggtgctgca gctgcgcggt gccggtggcc tgccgtcggc ggcgatcggt 360
ggcggcgtcg cggttggtga gcccgtcgtc gcgatgggca acagcggtgg gcagggcgga 420
acgccccgtg cggtgcctgg cagggtggtc gcgctcggcc aaaccgtgca ggcgtcggat 480
tcgctgaccg gtgccgaaga gacattgaac gggttgatcc agttcgatgc cgcgatccag 540
cccggtgatg cgggcgggcc cgtcgtcaac ggcctaggac aggtggtcgg tatgaacacg 600
gccgcgtccg ataacttcca gctgtcccag ggtgggcagg gattcgccat tccgatcggg 660
caggcgatgg cgatcgcggg ccagatccga tcgggtgggg ggtcacccac cgttcatatc 720
gggcctaccg ccttcctcgg cttgggtgtt gtcgacaaca acggcaacgg cgcacgagtc 780
caacgcgtgg tcgggagcgc tccggcggca agtctcggca tctccaccgg cgacgtgatc 840
accgcggtcg acggcgctcc gatcaactcg gccaccgcga tggcggacgc gcttaacggg 900
catcatcccg gtgacgtcat ctcggtgacc tggcaaacca agtcgggcgg cacgcgtaca 960
gggaacgtga cattggccga gggacccccg gcctgagaat tc                    1002
```

<212> PRT
<213> Ra35FLMutSA
<400> SEQ ID NO:6

```
Met His His His His His His Ala Pro Pro Ala Leu Ser Gln Asp Arg
                  5                  10                  15

Phe Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala
             20                  25                  30

Gln Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn
         35                  40                  45

Asn Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val
     50                  55                  60

Val Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala
 65                  70                  75                  80

Phe Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr
                 85                  90                  95

Asp Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly
            100                 105                 110

Leu Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val
        115                 120                 125

Val Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val
    130                 135                 140

Pro Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser
145                 150                 155                 160

Leu Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala
                165                 170                 175

Ala Ile Gln Pro Gly Asp Ala Gly Gly Pro Val Val Asn Gly Leu Gly
            180                 185                 190

Gln Val Val Gly Met Asn Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser
        195                 200                 205

Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile
    210                 215                 220

Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly
225                 230                 235                 240

Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly
                245                 250                 255
```

Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly
260 265 270

Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn
275 280 285

Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp
290 295 300

Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly
305 310 315 320

Asn Val Thr Leu Ala Glu Gly Pro Pro Ala
325 330

(2) INFORMATION FOR SEQ ID NO:7: Ra35 (MTB32A N-term)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 615 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

gccccgccggccttgtcgcaggaccggttcgccgacttccccgcgctgcccctcgacccgtccgcg
atggtcgcccaagtggggccacaggtggtcaacatcaacaccaaactgggctacaacaacgccgtg
ggcgccgggaccggcatcgtcatcgatcccaacggtgtcgtgctgaccaacaaccacgtgatcgcg
ggcgccaccgacatcaatgcgttcagcgtcggctccggccaaacctacggcgtcgatgtggtcggg
tatgaccgcacccaggatgtcgcggtgctgcagctgcgcggtgccggtggcctgccgtcggcggcg
atcggtggcggcgtcgcggttggtgagcccgtcgtcgcgatgggcaacagcggtgggcagggcgga
acgccccgtgcggtgcctggcagggtggtcgcgctcggccaaaccgtgcaggcgtcggattcgctg
accggtgccgaagagacattgaacgggttgatccagttcgatgccgcgatccagcccggtgaggcg
ggcgggcccgtcgtcaacggcctaggacaggtggtcggtatgaacacggccgcgtcc

(2) INFORMATION FOR SEQ ID NO:8: Ra35 (MTB32A N-term)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 205 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu

Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Ala Pro Gln Val Val

Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr

Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val

Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln

Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala

Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly

Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly

Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu

Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr

Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser

Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr

Ala Ala Ser

(2) INFORMATION FOR SEQ ID NO:9: Ra12

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 447 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CGGTATGAAC ACGGCCGCGT CCGATAACTT CCAGCTGTCC CAGGGTGGGC AGGGATTCGC      60
CATTCCGATC GGGCAGGCGA TGGCGATCGC GGGCCAGATC CGATCGGGTG GGGGGTCACC     120
CACCGTTCAT ATCGGGCCTA CCGCCTTCCT CGGCTTGGGT GTTGTCGACA ACAACGGCAA     180
CGGCGCACGA GTCCAACGCG TGGTCGGGAG CGCTCCGGCG GCAAGTCTCG GCATCTCCAC     240
CGGCGACGTG ATCACCGCGG TCGACGGCGC TCCGATCAAC TCGGCCACCG CGATGGCGGA     300
CGCGCTTAAC GGGCATCATC CCGGTGACGT CATCTCGGTG AACTGGCAAA CCAAGTCGGG     360
CGGCACGCGT ACAGGGAACG TGACATTGGC CGAGGGACCC CCGGCCTGAT TTCGTCGYGG     420
ATACCACCCG CCGGCCGGCC AATTGGA                                         447
```

(2) INFORMATION FOR SEQ ID NO:10: Ra12

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe
1               5                  10                  15
Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser
            20                  25                  30
Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly
        35                  40                  45
Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val
    50                  55                  60
Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val
65                  70                  75                  80
Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala
                85                  90                  95
Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Asn Trp
            100                 105                 110
Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu
        115                 120                 125
Gly Pro Pro Ala
    130
```

(2) INFORMATION FOR SEQ ID NO:11: TbH9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 851 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
CTGCAGGGTG GCGTGGATGA GCGTCACCGC GGGGCAGGCC GAGCTGACCG CCGCCCAGGT      60
CCGGGTTGCT GCGGCGGCCT ACGAGACGGC GTATGGGCTG ACGGTGCCCC CGCCGGTGAT     120
```

```
CGCCGAGAAC CGTGCTGAAC TGATGATTCT GATAGCGACC AACCTCTTGG GGCAAAACAC     180
CCCGGCGATC GCGGTCAACG AGGCCGAATA CGGCGAGATG TGGGCCCAAG ACGCCGCCGC     240
GATGTTTGGC TACGCCGCGG CGACGGCGAC GGCGACGGCG ACGTTGCTGC CGTTCGAGGA     300
GGCGCCGGAG ATGACCAGCG CGGGTGGGCT CCTCGAGCAG GCCGCCGCGG TCGAGGAGGC     360
CTCCGACACC GCCGCGGCGA ACCAGTTGAT GAACAATGTG CCCCAGGCGC TGAAACAGTT     420
GGCCCAGCCC ACGCAGGGCA CCACGCCTTC TTCCAAGCTG GGTGGCCTGT GGAAGACGGT     480
CTCGCCGCAT CGGTCGCCGA TCAGCAACAT GGTGTCGATG GCCAACAACC ACATGTCGAT     540
GACCAACTCG GGTGTGTCGA TGACCAACAC CTTGAGCTCG ATGTTGAAGG GCTTTGCTCC     600
GGCGGCGGCC GCCCAGGCCG TGCAAACCGC GGCGCAAAAC GGGGTCCGGG CGATGAGCTC     660
GCTGGGCAGC TCGCTGGGTT CTTCGGGTCT GGGCGGTGGG GTGGCCGCCA ACTTGGGTCG     720
GGCGGCCTCG GTACGGTATG GTCACCGGGA TGGCGGAAAA TATGCANAGT CTGGTCGGCG     780
GAACGGTGGT CCGGCGTAAG GTTTACCCCC GTTTTCTGGA TGCGGTGAAC TTCGTCAACG     840
GAAACAGTTA C                                                          851
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 263 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12: TbH9

```
Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala Ala
1               5                  10                  15
Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu Thr
            20                  25                  30
Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile Leu
        35                  40                  45
Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val Asn
    50                  55                  60
Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met Phe
65                  70                  75                  80
Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu Pro Phe
                85                  90                  95
Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln Ala
            100                 105                 110
Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln Leu Met
        115                 120                 125
Asn Asn Val Pro Gln Ala Leu Lys Gln Leu Ala Gln Pro Thr Gln Gly
    130                 135                 140
Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val Ser Pro
145                 150                 155                 160
His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn His Met
                165                 170                 175
Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser Ser Met
            180                 185                 190
Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala Gln Ala Val Gln Thr Ala
        195                 200                 205
Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser Leu Gly
    210                 215                 220
Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg Ala Ala
225                 230                 235                 240
Ser Val Arg Tyr Gly His Arg Asp Gly Gly Lys Tyr Ala Xaa Ser Gly
                245                 250                 255
Arg Arg Asn Gly Gly Pro Ala
            260
```

(2) INFORMATION FOR SEQ ID NO:13: TBH9FL

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3058 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCGTACCC GTGCGAGTGC TCGGGCCGTT TGAGGATGGA GTGCACGTGT CTTTCGTGAT 60

GGCATACCCA GAGATGTTGG CGGCGGCGGC TGACACCCTG CAGAGCATCG GTGCTACCAC 120

TGTGGCTAGC AATGCCGCTG CGGCGGCCCC GACGACTGGG GTGGTGCCCC CCGCTGCCGA 180

TGAGGTGTCG GCGCTGACTG CGGCGCACTT CGCCGCACAT GCGGCGATGT ATCAGTCCGT 240

GAGCGCTCGG GCTGCTGCGA TTCATGACCA GTTCGTGGCC ACCCTTGCCA GCAGCGCCAG 300

CTCGTATGCG GCCACTGAAG TCGCCAATGC GGCGGCGGCC AGCTAAGCCA GGAACAGTCG 360

GCACGAGAAA CCACGAGAAA TAGGGACACG TAATGGTGGA TTTCGGGGCG TTACCACCGG 420

AGATCAACTC CGCGAGGATG TACGCCGGCC CGGGTTCGGC CTCGCTGGTG GCCGCGGCTC 480

AGATGTGGGA CAGCGTGGCG AGTGACCTGT TTTCGGCCGC GTCGGCGTTT CAGTCGGTGG 540

TCTGGGGTCT GACGGTGGGG TCGTGGATAG GTTCGTCGGC GGGTCTGATG GTGGCGGCGG 600

CCTCGCCGTA TGTGGCGTGG ATGAGCGTCA CCGCGGGGCA GGCCGAGCTG ACCGCCGCCC 660

AGGTCCGGGT TGCTGCGGCG GCCTACGAGA CGGCGTATGG GCTGACGGTG CCCCCGCCGG 720

TGATCGCCGA GAACCGTGCT GAACTGATGA TTCTGATAGC GACCAACCTC TTGGGGCAAA 780

ACACCCCGGC GATCGCGGTC AACGAGGCCG AATACGGCGA GATGTGGGCC CAAGACGCCG 840

CCGCGATGTT TGGCTACGCC GCGGCGACGG CGACGGCGAC GGCGACGTTG CTGCCGTTCG 900

AGGAGGCGCC GGAGATGACC AGCGCGGGTG GGCTCCTCGA GCAGGCCGCC GCGGTCGAGG 960

AGGCCTCCGA CACCGCCGCG GCGAACCAGT TGATGAACAA TGTGCCCCAG GCGCTGCAAC 1020

AGCTGGCCCA GCCCACGCAG GGCACCACGC CTTCTTCCAA GCTGGGTGGC CTGTGGAAGA 1080

CGGTCTCGCC GCATCGGTCG CCGATCAGCA ACATGGTGTC GATGGCCAAC AACCACATGT 1140

CGATGACCAA CTCGGGTGTG TCGATGACCA ACACCTTGAG CTCGATGTTG AAGGGCTTTG 1200

CTCCGGCGGC GGCCGCCCAG GCCGTGCAAA CCGCGGCGCA AAACGGGGTC CGGGCGATGA 1260

GCTCGCTGGG CAGCTCGCTG GGTTCTTCGG GTCTGGGCGG TGGGGTGGCC GCCAACTTGG 1320

GTCGGGCGGC CTCGGTCGGT TCGTTGTCGG TGCCGCAGGC CTGGGCCGCG GCCAACCAGG 1380

CAGTCACCCC GGCGGCGCGG GCGCTGCCGC TGACCAGCCT GACCAGCGCC GCGGAAAGAG 1440

GGCCCGGGCA GATGCTGGGC GGGCTGCCGG TGGGGCAGAT GGGCGCCAGG GCCGGTGGTG 1500

GGCTCAGTGG TGTGCTGCGT GTTCCGCCGC GACCCTATGT GATGCCGCAT TCTCCGGCGG 1560

CCGGCTAGGA GAGGGGGCGC AGACTGTCGT TATTTGACCA GTGATCGGCG GTCTCGGTGT 1620

TTCCGCGGCC GGCTATGACA ACAGTCAATG TGCATGACAA GTTACAGGTA TTAGGTCCAG 1680

GTTCAACAAG GAGACAGGCA ACATGGCCTC ACGTTTTATG ACGGATCCGC ACGCGATGCG 1740

GGACATGGCG GGCCGTTTTG AGGTGCACGC CCAGACGGTG GAGGACGAGG CTCGCCGGAT 1800

GTGGGCGTCC GCGCAAAACA TTTCCGGTGC GGGCTGGAGT GGCATGGCCG AGGCGACCTC 1860

GCTAGACACC ATGGCCCAGA TGAATCAGGC GTTTCGCAAC ATCGTGAACA TGCTGCACGG 1920

GGTGCGTGAC GGGCTGGTTC GCGACGCCAA CAACTACGAG CAGCAAGAGC AGGCCTCCCA 1980

GCAGATCCTC AGCAGCTAAC GTCAGCCGCT GCAGCACAAT ACTTTTACAA GCGAAGGAGA 2040

ACAGGTTCGA TGACCATCAA CTATCAATTC GGGGATGTCG ACGCTCACGG CGCCATGATC 2100

CGCGCTCAGG CCGGGTTGCT GGAGGCCGAG CATCAGGCCA TCATTCGTGA TGTGTTGACC 2160

GCGAGTGACT TTTGGGGCGG CGCCGGTTCG GCGGCCTGCC AGGGGTTCAT TACCCAGTTG 2220

GGCCGTAACT TCCAGGTGAT CTACGAGCAG GCCAACGCCC ACGGGCAGAA GGTGCAGGCT 2280

GCCGGCAACA ACATGGCGCA AACCGACAGC GCCGTCGGCT CCAGCTGGGC CTGACACCAG 2340

GCCAAGGCCA GGGACGTGGT GTACGAGTGA AGTTCCTCGC GTGATCCTTC GGGTGGCAGT 2400

CTAAGTGGTC AGTGCTGGGG TGTTGGTGGT TTGCTGCTTG GCGGGTTCTT CGGTGCTGGT 2460

CAGTGCTGCT CGGGCTCGGG TGAGGACCTC GAGGCCCAGG TAGCGCCGTC CTTCGATCCA 2520

TTCGTCGTGT TGTTCGGCGA GGACGGCTCC GACGAGGCGG ATGATCGAGG CGCGGTCGGG 2580

GAAGATGCCC ACGACGTCGG TTCGGCGTCG TACCTCTCGG TTGAGGCGTT CCTGGGGGTT 2640

GTTGGACCAG ATTTGGCGCC AGATCTGCTT GGGGAAGGCG GTGAACGCCA GCAGGTCGGT 2700

GCGGGCGGTG TCGAGGTGCT CGGCCACCGC GGGGAGTTTG TCGGTCAGAG CGTCGAGTAC 2760

CCGATCATAT TGGGCAACAA CTGATTCGGC GTCGGGCTGG TCGTAGATGG AGTGCAGCAG 2820

GGTGCGCACC CACGGCCAGG AGGGCTTCGG GGTGGCTGCC ATCAGATTGG CTGCGTAGTG 2880

GGTTCTGCAG CGCTGCCAGG CCGCTGCGGG CAGGGTGGCG CCGATCGCGG CCACCAGGCC 2940

GGCGTGGGCG TCGCTGGTGA CCAGCGCGAC CCCGGACAGG CCGCGGGCGA CCAGGTCGCG 3000

GAAGAACGCC AGCCAGCCGG CCCCGTCCTC GGCGGAGGTG ACCTGGATGC CCAGGATC 3058

(2) INFORMATION FOR SEQ ID NO:14: TbH9FL

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 391 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Val Asp Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met
1 5 10 15

Tyr Ala Gly Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp
20 25 30

Asp Ser Val Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser
35 40 45

Val Val Trp Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly
50 55 60

Leu Met Val Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr
65 70 75 80

Ala Gly Gln Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala
85 90 95

Ala Tyr Glu Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala
100 105 110

```
Glu Asn Arg Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly
        115                 120                 125

Gln Asn Thr Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met
    130                 135                 140

Trp Ala Gln Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala
145                 150                 155                 160

Thr Ala Thr Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr
                165                 170                 175

Ser Ala Gly Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser
            180                 185                 190

Asp Thr Ala Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu
        195                 200                 205

Gln Gln Leu Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu
    210                 215                 220

Gly Gly Leu Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn
225                 230                 235                 240

Met Val Ser Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val
                245                 250                 255

Ser Met Thr Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala
            260                 265                 270

Ala Ala Ala Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala
        275                 280                 285

Met Ser Ser Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly
    290                 295                 300

Val Ala Ala Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val
305                 310                 315                 320

Pro Gln Ala Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg
                325                 330                 335

Ala Leu Pro Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly
            340                 345                 350

Gln Met Leu Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly
        355                 360                 365

Gly Gly Leu Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met
    370                 375                 380

Pro His Ser Pro Ala Ala Gly
385                 390
```

<210> SEQ ID NO:15
<211> 2287
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:tri-fusion protein Mtb72F(Ra12-TbH9-Ra35 or Mtb32-Mtb39 fusion)

```
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                                                Met His His His His
                                                  1               5
```

```
cat cac acg gcc gcg tcc gat aac ttc cag ctg tcc cag ggt ggg cag   104
His His Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln
                10                  15                  20

gga ttc gcc att ccg atc ggg cag gcg atg gcg atc gcg ggc cag atc   152
Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile
            25                  30                  35

cga tcg ggt ggg ggg tca ccc acc gtt cat atc ggg cct acc gcc ttc   200
Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe
        40                  45                  50

ctc ggc ttg ggt gtt gtc gac aac aac ggc aac ggc gca cga gtc caa   248
Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln
    55                  60                  65

cgc gtg gtc ggg agc gct ccg gcg gca agt ctc ggc atc tcc acc ggc   296
Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly
70                  75                  80                  85

gac gtg atc acc gcg gtc gac ggc gct ccg atc aac tcg gcc acc gcg   344
Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala
                90                  95                  100

atg gcg gac gcg ctt aac ggg cat cat ccc ggt gac gtc atc tcg gtg   392
Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val
            105                 110                 115

acc tgg caa acc aag tcg ggc ggc acg cgt aca ggg aac gtg aca ttg   440
Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu
        120                 125                 130

gcc gag gga ccc ccg gcc gaa ttc atg gtg gat ttc ggg gcg tta cca   488
Ala Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu Pro
    135                 140                 145

ccg gag atc aac tcc gcg agg atg tac gcc ggc ccg ggt tcg gcc tcg   536
Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser
150                 155                 160                 165

ctg gtg gcc gcg gct cag atg tgg gac agc gtg gcg agt gac ctg ttt   584
Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe
                170                 175                 180

tcg gcc gcg tcg gcg ttt cag tcg gtg gtc tgg ggt ctg acg gtg ggg   632
Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly
            185                 190                 195

tcg tgg ata ggt tcg tcg gcg ggt ctg atg gtg gcg gcg gcc tcg ccg   680
Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro
        200                 205                 210

tat gtg gcg tgg atg agc gtc acc gcg ggg cag gcc gag ctg acc gcc   728
Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala
    215                 220                 225

gcc cag gtc cgg gtt gct gcg gcg gcc tac gag acg gcg tat ggg ctg   776
Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu
230                 235                 240                 245

acg gtg ccc ccg ccg gtg atc gcc gag aac cgt gct gaa ctg atg att   824
Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile
                250                 255                 260

ctg ata gcg acc aac ctc ttg ggg caa aac acc ccg gcg atc gcg gtc   872
Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val
            265                 270                 275
```

```
aac gag gcc gaa tac ggc gag atg tgg gcc caa gac gcc gcc gcg atg   920
Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met
        280                 285                 290

ttt ggc tac gcc gcg gcg acg gcg acg gcg acg gcg acg ttg ctg ccg   968
Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu Pro
    295                 300                 305

ttc gag gag gcg ccg gag atg acc agc gcg ggt ggg ctc ctc gag cag   1016
Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln
310                 315                 320                 325

gcc gcc gcg gtc gag gag gcc tcc gac acc gcc gcg gcg aac cag ttg   1064
Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln Leu
                330                 335                 340

atg aac aat gtg ccc cag gcg ctg caa cag ctg gcc cag ccc acg cag   1112
Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu Ala Gln Pro Thr Gln
            345                 350                 355

ggc acc acg cct tct tcc aag ctg ggt ggc ctg tgg aag acg gtc tcg   1160
Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val Ser
        360                 365                 370

ccg cat cgg tcg ccg atc agc aac atg gtg tcg atg gcc aac aac cac   1208
Pro His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn His
    375                 380                 385

atg tcg atg acc aac tcg ggt gtg tcg atg acc aac acc ttg agc tcg   1256
Met Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser Ser
390                 395                 400                 405

atg ttg aag ggc ttt gct ccg gcg gcg gcc cgc cag gcc gtg caa acc   1304
Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Arg Gln Ala Val Gln Thr
                410                 415                 420

gcg gcg caa aac ggg gtc cgg gcg atg agc tcg ctg ggc agc tcg ctg   1352
Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser Leu
            425                 430                 435

ggt tct tcg ggt ctg ggc ggt ggg gtg gcc gcc aac ttg ggt cgg gcg   1400
Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg Ala
        440                 445                 450

gcc tcg gtc ggt tcg ttg tcg gtg ccg cag gcc tgg gcc gcg gcc aac   1448
Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala Trp Ala Ala Ala Asn
    455                 460                 465

cag gca gtc acc ccg gcg gcg cgg gcg ctg ccg ctg acc agc ctg acc   1496
Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu Thr
470                 475                 480                 485

agc gcc gcg gaa aga ggg ccc ggg cag atg ctg ggc ggg ctg ccg gtg   1544
Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu Gly Gly Leu Pro Val
                490                 495                 500

ggg cag atg ggc gcc agg gcc ggt ggt ggg ctc agt ggt gtg ctg cgt   1592
Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu Ser Gly Val Leu Arg
            505                 510                 515

gtt ccg ccg cga ccc tat gtg atg ccg cat tct ccg gca gcc ggc gat   1640
Val Pro Pro Arg Pro Tyr Val Met Pro His Ser Pro Ala Ala Gly Asp
        520                 525                 530

atc gcc ccg ccg gcc ttg tcg cag gac cgg ttc gcc gac ttc ccc gcg   1688
Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala
    535                 540                 545
```

```
ctg ccc ctc gac ccg tcc gcg atg gtc gcc caa gtg ggg cca cag gtg   1736
Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val
550                 555                 560                 565

gtc aac atc aac acc aaa ctg ggc tac aac aac gcc gtg ggc gcc ggg   1784
Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly
                570                 575                 580

acc ggc atc gtc atc gat ccc aac ggt gtc gtg ctg acc aac aac cac   1832
Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His
            585                 590                 595

gtg atc gcg ggc gcc acc gac atc aat gcg ttc agc gtc ggc tcc ggc   1880
Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly
        600                 605                 610

caa acc tac ggc gtc gat gtg gtc ggg tat gac cgc acc cag gat gtc   1928
Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val
    615                 620                 625

gcg gtg ctg cag ctg cgc ggt gcc ggt ggc ctg ccg tcg gcg gcg atc   1976
Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile
630                 635                 640                 645

ggt ggc ggc gtc gcg gtt ggt gag ccc gtc gtc gcg atg ggc aac agc   2024
Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser
                650                 655                 660

ggt ggg cag ggc gga acg ccc cgt gcg gtg cct ggc agg gtg gtc gcg   2072
Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala
            665                 670                 675

ctc ggc caa acc gtg cag gcg tcg gat tcg ctg acc ggt gcc gaa gag   2120
Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu
        680                 685                 690

aca ttg aac ggg ttg atc cag ttc gat gcc gcg atc cag ccc ggt gat   2168
Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp
    695                 700                 705

tcg ggc ggg ccc gtc gtc aac ggc cta gga cag gtg gtc ggt atg aac   2216
Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn
710                 715                 720                 725

acg gcc gcg tcc taggatatcc atcacactgg cggccgctcg agcagatccg       2268
Thr Ala Ala Ser

gntgtaacaa agcccgaaa                                              2287
```

<210> SEQ ID NO:16
<211> 729
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:tri-fusion protein Mtb72F (Ra12-TbH9-Ra35 or Mtb32-Mtb39 fusion)

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Met His His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
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Ser Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
            20                  25                  30

Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
        35                  40                  45

Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
```

```
     50                  55                  60

Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
 65                  70                  75                  80

Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
                 85                  90                  95

Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
            100                 105                 110

Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr
        115                 120                 125

Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala Glu Phe Met Val Asp
    130                 135                 140

Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly
145                 150                 155                 160

Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val
                165                 170                 175

Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp
            180                 185                 190

Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val
        195                 200                 205
Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln
    210                 215                 220

Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu
225                 230                 235                 240

Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg
                245                 250                 255

Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr
            260                 265                 270

Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln
        275                 280                 285

Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr
    290                 295                 300

Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly
305                 310                 315                 320

Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala
                325                 330                 335

Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu
            340                 345                 350

Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu
        355                 360                 365

Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn Met Val Ser
    370                 375                 380

Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr
385                 390                 395                 400

Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Arg
                405                 410                 415

Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser
```

```
                420                 425                 430

Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala
        435                 440                 445

Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala
    450                 455                 460

Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro
465                 470                 475                 480

Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu
                485                 490                 495

Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu
            500                 505                 510

Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser
        515                 520                 525

Pro Ala Ala Gly Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe
    530                 535                 540

Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln
545                 550                 555                 560

Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn
                565                 570                 575

Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val
            580                 585                 590

Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe
        595                 600                 605

Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp
    610                 615                 620

Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu
625                 630                 635                 640

Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val
                645                 650                 655

Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro
            660                 665                 670

Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu
        675                 680                 685

Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala
    690                 695                 700

Ile Gln Pro Gly Asp Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln
705                 710                 715                 720

Val Val Gly Met Asn Thr Ala Ala Ser
                725
```

<210> SEQ ID NO:17
<211> 2190
<212> DNA
<213> Mtb72FMutSA

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ggggggtcac ccaccgttca tatcgggcct accgccttcc tcggcttggg tgttgtcgac 180
```

```
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ccttcttcca agctgggtgg cctgtggaag acggtctcgc cgcatcggtc gccgatcagc 1140
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ccgtcggcgg cgatcggtgg cggcgtcgcg gttggtgagc ccgtcgtcgc gatgggcaac 1980
agcggtgggc agggcggaac gccccgtgcg gtgcctggca gggtggtcgc gctcggccaa 2040
accgtgcagg cgtcggattc gctgaccggt gccgaagaga cattgaacgg gttgatccag 2100
ttcgatgccg cgatccagcc cggtgatgcg ggcgggcccg tcgtcaacgg cctaggacag 2160
gtggtcggta tgaacacggc cgcgtcctag                                  2190
```

<210> SEQ ID NO:18
<211> 729
<212> PRT
<213> Mtb72FMutSA

```
Met His His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
                  5                  10                  15

Ser Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
             20                  25                  30

Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
         35                  40                  45

Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
     50                  55                  60

Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
 65                  70                  75                  80

Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
                 85                  90                  95

Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
            100                 105                 110

Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr
        115                 120                 125

Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala Glu Phe Met Val Asp
    130                 135                 140
```

```
Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly
145                 150                 155                 160

Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val
                165                 170                 175

Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp
            180                 185                 190

Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val
        195                 200                 205

Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln
    210                 215                 220

Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu
225                 230                 235                 240

Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg
                245                 250                 255

Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr
            260                 265                 270

Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln
        275                 280                 285

Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr
    290                 295                 300

Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly
305                 310                 315                 320

Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala
                325                 330                 335

Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu
            340                 345                 350

Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu
        355                 360                 365

Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn Met Val Ser
    370                 375                 380

Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr
385                 390                 395                 400

Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala
                405                 410                 415

Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser
            420                 425                 430

Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala
        435                 440                 445

Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala
    450                 455                 460

Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro
465                 470                 475                 480

Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu
                485                 490                 495

Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu
            500                 505                 510
```

```
Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser
        515                 520                 525

Pro Ala Ala Gly Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe
    530                 535                 540

Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln
545                 550                 555                 560

Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn
                565                 570                 575

Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val
            580                 585                 590

Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe
        595                 600                 605

Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp
    610                 615                 620

Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu
625                 630                 635                 640

Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val
                645                 650                 655

Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro
            660                 665                 670

Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu
        675                 680                 685

Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala
    690                 695                 700

Ile Gln Pro Gly Asp Ala Gly Gly Pro Val Val Asn Gly Leu Gly Gln
705                 710                 715                 720

Val Val Gly Met Asn Thr Ala Ala Ser
                725
```

<210> SEQ ID NO:19
<211> 1797
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:bi-fusion protein TbH9-Ra35 (designated Mtb59f)
<222> (1)..(1791)

```
cat atg cat cac cat cac cat cac atg gtg gat ttc ggg gcg tta cca   48
His Met His His His His His His Met Val Asp Phe Gly Ala Leu Pro
 1               5                  10                  15

ccg gag atc aac tcc gcg agg atg tac gcc ggc ccg ggt tcg gcc tcg   96
Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser
            20                  25                  30

ctg gtg gcc gcg gct cag atg tgg gac agc gtg gcg agt gac ctg ttt   144
Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe
        35                  40                  45

tcg gcc gcg tcg gcg ttt cag tcg gtg gtc tgg ggt ctg acg gtg ggg   192
Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly
    50                  55                  60
```

```
tcg tgg ata ggt tcg tcg gcg ggt ctg atg gtg gcg gcg gcc tcg ccg   240
Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro
 65                  70                  75                  80

tat gtg gcg tgg atg agc gtc acc gcg ggg cag gcc gag ctg acc gcc   288
Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala
                85                  90                  95

gcc cag gtc cgg gtt gct gcg gcg gcc tac gag acg gcg tat ggg ctg   336
Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu
            100                 105                 110

acg gtg ccc ccg ccg gtg atc gcc gag aac cgt gct gaa ctg atg att   384
Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile
        115                 120                 125

ctg ata gcg acc aac ctc ttg ggg caa aac acc ccg gcg atc gcg gtc   432
Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val
    130                 135                 140

aac gag gcc gaa tac ggc gag atg tgg gcc caa gac gcc gcc gcg atg   480
Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met
145                 150                 155                 160

ttt ggc tac gcc gcg gcg acg gcg acg gcg acg gcg acg ttg ctg ccg   528
Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu Pro
                165                 170                 175

ttc gag gag gcg ccg gag atg acc agc gcg ggt ggg ctc ctc gag cag   576
Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln
            180                 185                 190

gcc gcc gcg gtc gag gag gcc tcc gac acc gcc gcg gcg aac cag ttg   624
Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln Leu
        195                 200                 205

atg aac aat gtg ccc cag gcg ctg caa cag ctg gcc cag ccc acg cag   672
Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu Ala Gln Pro Thr Gln
    210                 215                 220

ggc acc acg cct tct tcc aag ctg ggt ggc ctg tgg aag acg gtc tcg   720
Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val Ser
225                 230                 235                 240

ccg cat cgg tcg ccg atc agc aac atg gtg tcg atg gcc aac aac cac   768
Pro His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn His
                245                 250                 255

atg tcg atg acc aac tcg ggt gtg tcg atg acc aac acc ttg agc tcg   816
Met Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser Ser
            260                 265                 270

atg ttg aag ggc ttt gct ccg gcg gcg gcc gcc cag gcc gtg caa acc   864
Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala Gln Ala Val Gln Thr
        275                 280                 285

gcg gcg caa aac ggg gtc cgg gcg atg agc tcg ctg ggc agc tcg ctg   912
Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser Leu
    290                 295                 300

ggt tct tcg ggt ctg ggc ggt ggg gtg gcc gcc aac ttg ggt cgg gcg   960
Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg Ala
305                 310                 315                 320

gcc tcg gtc ggt tcg ttg tcg gtg ccg cag gcc tgg gcc gcg gcc aac  1008
Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala Trp Ala Ala Ala Asn
                325                 330                 335
```

```
cag gca gtc acc ccg gcg gcg cgg gcg ctg ccg ctg acc agc ctg acc   1056
Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu Thr
            340                 345                 350

agc gcc gcg gaa aga ggg ccc ggg cag atg ctg ggc ggg ctg ccg gtg   1104
Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu Gly Gly Leu Pro Val
        355                 360                 365

ggg cag atg ggc gcc agg gcc ggt ggt ggg ctc agt ggt gtg ctg cgt   1152
Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu Ser Gly Val Leu Arg
    370                 375                 380

gtt ccg ccg cga ccc tat gtg atg ccg cat tct ccg gca gcc ggc gat   1200
Val Pro Pro Arg Pro Tyr Val Met Pro His Ser Pro Ala Ala Gly Asp
385                 390                 395                 400

atc gcc ccg ccg gcc ttg tcg cag gac cgg ttc gcc gac ttc ccc gcg   1248
Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala
                405                 410                 415

ctg ccc ctc gac ccg tcc gcg atg gtc gcc caa gtg ggg cca cag gtg   1296
Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val
            420                 425                 430

gtc aac atc aac acc aaa ctg ggc tac aac aac gcc gtg ggc gcc ggg   1344
Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly
        435                 440                 445

acc ggc atc gtc atc gat ccc aac ggt gtc gtg ctg acc aac aac cac   1392
Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His
    450                 455                 460

gtg atc gcg ggc gcc acc gac atc aat gcg ttc agc gtc ggc tcc ggc   1440
Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly
465                 470                 475                 480

caa acc tac ggc gtc gat gtg gtc ggg tat gac cgc acc cag gat gtc   1488
Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val
                485                 490                 495

gcg gtg ctg cag ctg cgc ggt gcc ggt ggc ctg ccg tcg gcg gcg atc   1536
Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile
            500                 505                 510

ggt ggc ggc gtc gcg gtt ggt gag ccc gtc gtc gcg atg ggc aac agc   1584
Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser
        515                 520                 525

ggt ggg cag ggc gga acg ccc cgt gcg gtg cct ggc agg gtg gtc gcg   1632
Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala
    530                 535                 540

ctc ggc caa acc gtg cag gcg tcg gat tcg ctg acc ggt gcc gaa gag   1680
Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu
545                 550                 555                 560

aca ttg aac ggg ttg atc cag ttc gat gcc gcg atc cag ccc ggt gat   1728
Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp
                565                 570                 575

tcg ggc ggg ccc gtc gtc aac ggc cta gga cag gtg gtc ggt atg aac   1776
Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn
            580                 585                 590

acg gcc gcg tcc taggatatc                                        1797
Thr Ala Ala Ser
        595
```

<210> SEQ ID NO:20
<211> 596
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:bi-fusion protein TbH9-Ra35 (designated Mtb59f)

```
His Met His His His His His His Met Val Asp Phe Gly Ala Leu Pro
 1               5                  10                  15

Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser
            20                  25                  30

Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe
        35                  40                  45

Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly
    50                  55                  60

Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro
65                  70                  75                  80

Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala
                85                  90                  95

Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu
            100                 105                 110

Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile
        115                 120                 125

Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val
    130                 135                 140

Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met
145                 150                 155                 160

Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu Pro
                165                 170                 175

Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln
            180                 185                 190

Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln Leu
        195                 200                 205

Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu Ala Gln Pro Thr Gln
    210                 215                 220

Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val Ser
225                 230                 235                 240
Pro His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn His
                245                 250                 255

Met Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser Ser
            260                 265                 270

Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala Gln Ala Val Gln Thr
        275                 280                 285

Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser Leu
    290                 295                 300

Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg Ala
305                 310                 315                 320

Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala Trp Ala Ala Ala Asn
```

325 330 335

Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu Thr
340 345 350

Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu Gly Gly Leu Pro Val
355 360 365

Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu Ser Gly Val Leu Arg
370 375 380

Val Pro Pro Arg Pro Tyr Val Met Pro His Ser Pro Ala Ala Gly Asp
385 390 395 400

Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala
405 410 415

Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val
420 425 430

Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly
435 440 445

Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His
450 455 460

Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly
465 470 475 480

Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val
485 490 495

Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile
500 505 510

Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser
515 520 525

Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala
530 535 540

Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu
545 550 555 560

Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp
565 570 575

Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn
580 585 590

Thr Ala Ala Ser
595

(2) INFORMATION FOR SEQ ID NO:21: DPV (MTB8.4)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGTGGCAATG TCGTTGACCG TCGGGGCCGG GGTCGCCTCC GCAGATCCCG TGGACGCGGT 60
CATTAACACC ACCTGCAATT ACGGGCAGGT AGTAGCTGCG CTCAACGCGA CGGATCCGGG 120
GGCTGCCGCA CAGTTCAACG CCTCACCGGT GGCGCAGTCC TATTTGCGCA ATTTCCTCGC 180
CGCACCGCCA CCTCAGCGCG CTGCCATGGC CGCGCAATTG CAAGCTGTGC CGGGGGCGGC 240

```
ACAGTACATC GGCCTTGTCG AGTCGGTTGC CGGCTCCTGC AACAACTATT AAGCCCATGC     300
GGGCCCCATC CCGCGACCCG GCATCGTCGC CGGGGCTAGG CCAGATTGCC CCGCTCCTCA     360
ACGGGCCGCA TCCCGCGACC CGGCATCGTC GCCGGGGCTA GGCCAGATTG CCCCGCTCCT     420
CAACGGGCCG CATCTCGTGC CGAATTCCTG CAGCCCGGGG GATCCACTAG TTCTAGAGCG     480
GCCGCCACCG CGGTGGAGCT                                                 500
```

(2) INFORMATION FOR SEQ ID NO:22: DPV (MTB8.4)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Val Ala Met Ser Leu Thr Val Gly Ala Gly Val Ala Ser Ala Asp Pro
1               5                  10                  15
Val Asp Ala Val Ile Asn Thr Thr Cys Asn Tyr Gly Gln Val Val Ala
            20                  25                  30
Ala Leu Asn Ala Thr Asp Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser
        35                  40                  45
Pro Val Ala Gln Ser Tyr Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro
    50                  55                  60
Gln Arg Ala Ala Met Ala Ala Gln Leu Gln Ala Val Pro Gly Ala Ala
65                  70                  75                  80
Gln Tyr Ile Gly Leu Val Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:23: MSL (MTB9.8)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 585 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
TGGATTCCGA TAGCGGTTTC GGCCCCTCGA CGGGCGACCA CGGCGCGCAG GCCTCCGAAC      60
GGGGGGCCGG GACGCTGGGA TTCGCCGGGA CCGCAACCAA AGAACGCCGG GTCCGGGCGG     120
TCGGGCTGAC CGCACTGGCC GGTGATGAGT TCGGCAACGG CCCCCGGATG CCGATGGTGC     180
CGGGGACCTG GGAGCAGGGC AGCAACGAGC CCGAGGCGCC CGACGGATCG GGGAGAGGGG     240
GAGGCGACGG CTTACCGCAC GACAGCAAGT AACCGAATTC CGAATCACGT GGACCCGTAC     300
GGGTCGAAAG GAGAGATGTT ATGAGCCTTT TGGATGCTCA TATCCCACAG TTGGTGGCCT     360
CCCAGTCGGC GTTTGCCGCC AAGGCGGGGC TGATGCGGCA CACGATCGGT CAGGCCGAGC     420
AGGCGGCGAT GTCGGCTCAG GCGTTTCACC AGGGGGAGTC GTCGGCGGCG TTTCAGGCCG     480
CCCATGCCCG GTTTGTGGCG GCGGCCGCCA AAGTCAACAC CTTGTTGGAT GTCGCGCAGG     540
CGAATCTGGG TGAGGCCGCC GGTACCTATG TGGCCGCCGA TGCTG                      585
```

(2) INFORMATION FOR SEQ ID NO:24: MSL (MTB9.8)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Met Ser Leu Leu Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser
 1               5                  10                  15
Ala Phe Ala Ala Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala
            20                  25                  30
Glu Gln Ala Ala Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser
        35                  40                  45
Ala Ala Phe Gln Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys
    50                  55                  60
Val Asn Thr Leu Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala
65                  70                  75                  80
Gly Thr Tyr Val Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly
                85                  90                  95
Phe
```

(2) INFORMATION FOR SEQ ID NO:25: MTI (MTB9.9A)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1742 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
CCGCTCTCTT TCAACGTCAT AAGTTCGGTG GGCCAGTCGG CCGCGCGTGC ATATGGCACC      60
AATAACGCGT GTCCCATGGA TACCCGGACC GCACGACGGT AGAGCGGATC AGCGCAGCCG     120
GTGCCGAACA CTACCGCGTC CACGCTCAGC CCTGCCGCGT TGCGGAAGAT CGAGCCCAGG     180
TTCTCATGGT CGTTAACGCC TTCCAACACT GCGACGGTGC GCGCCCCGGC GACCACCTGA     240
GCAACGCTCG GCTCCGGCAC CCGGCGCGCG GCTGCCAACA CCCCACGATT GAGATGGAAG     300
CCGATCACCC GTGCCATGAC ATCAGCCGAC GCTCGATAGT ACGGCGCGCC GACACCGGCC     360
AGATCATCCT TGAGCTCGGC CAGCCGGCGG TCGGTGCCGA ACAGCGCCAG CGGCGTGAAC     420
CGTGAGGCCA GCATGCGCTG CACCACCAGC ACACCCTCGG CGATCACCAA CGCCTTGCCG     480
GTCGGCAGAT CGGGACNACN GTCGATGCTG TTCAGGTCAC GGAAATCGTC GAGCCGTGGG     540
TCGTCGGGAT CGCAGACGTC CTGAACATCG AGGCCGTCGG GGTGCTGGGC ACAACGGCCT     600
TCGGTCACGG GCTTTCGTCG ACCAGAGCCA GCATCAGATC GGCGGCGCTG CGCAGGATGT     660
CACGCTCGCT GCGGTTCAGC GTCGCGAGCC GCTCAGCCAG CCACTCTTGC AGAGAGCCGT     720
TGCTGGGATT AATTGGGAGA GGAAGACAGC ATGTCGTTCG TGACCACACA GCCGGAAGCC     780
CTGGCAGCTG CGGCGGCGAA CCTACAGGGT ATTGGCACGA CAATGAACGC CCAGAACGCG     840
GCCGCGGCTG CTCCAACCAC CGGAGTAGTG CCCGCAGCCG CCGATGAAGT ATCAGCGCTG     900
ACCGCGGCTC AGTTTGCTGC GCACGCGCAG ATGTACCAAA CGGTCAGCGC CCAGGCCGCG     960
GCCATTCACG AAATGTTCGT GAACACGCTG GTGGCCAGTT CTGGCTCATA CGCGGCCACC    1020
GAGGCGGCCA ACGCAGCCGC TGCCGGCTGA ACGGGCTCGC ACGAACCTGC TGAAGGAGAG    1080
GGGGAACATC CGGAGTTCTC GGGTCAGGGG TTGCGCCAGC GCCCAGCCGA TTCAGNTATC    1140
GGCGTCCATA ACAGCAGACG ATCTAGGCAT TCAGTACTAA GGAGACAGGC AACATGGCCT    1200
CACGTTTTAT GACGGATCCG CATGCGATGC GGGACATGGC GGGCCGTTTT GAGGTGCACG    1260
CCCAGACGGT GGAGGACGAG GCTCGCCGGA TGTGGGCGTC CGCGCAAAAC ATTTCCGGTG    1320
CGGGCTGGAG TGGCATGGCC GAGGCGACCT CGCTAGACAC CATGACCTAG ATGAATCAGG    1380
CGTTTCGCAA CATCGTGAAC ATGCTGCACG GGGTGCGTGA CGGGCTGGTT CGCGACGCCA    1440
ACAANTACGA ACAGCAAGAG CAGGCCTCCC AGCAGATCCT GAGCAGNTAG CGCCGAAAGC    1500
CACAGCTGNG TACGNTTTCT CACATTAGGA GAACACCAAT ATGACGATTA ATTACCAGTT    1560
CGGGGACGTC GACGCTCATG GCGCCATGAT CCGCGCTCAG GCGGCGTCGC TTGAGGCGGA    1620
GCATCAGGCC ATCGTTCGTG ATGTGTTGGC CGCGGGTGAC TTTTGGGGCG GCGCCGGTTC    1680
GGTGGCTTGC CAGGAGTTCA TTACCCAGTT GGGCCGTAAC TTCCAGGTGA TCTACGAGCA    1740
GG                                                                   1742
```

(2) INFORMATION FOR SEQ ID NO:26: MTI (MTB9.9A)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2836 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTTGATTCCG TTCGCGGCGC CGCCGAAGAC CACCAACTCC GCTGGGGTGG TCGCACAGGC 60
GGTTGCGTCG GTCAGCTGGC CGAATCCCAA TGATTGGTGG CTCNGTGCGG TTGCTGGGCT 120
CGATTACCCC CACGGAAAGG ACGACGATCG TTCGTTTGCT CGGTCAGTCG TACTTGGCGA 180
CGGGCATGGC GCGGTTTCTT ACCTCGATCG CACAGCAGCT GACCTTCGGC CCAGGGGGCA 240
CAACGGCTGG CTCCGGCGGA GCCTGGTACC CAACGCCACA ATTCGCCGGC CTGGGTGCAG 300
GCCCGGCGGT GTCGGCGAGT TTGGCGCGGG CGGAGCCGGT CGGGAGGTTG TCGGTGCCGC 360
CAAGTTGGGC CGTCGCGGCT CCGGCCTTCG CGGAGAAGCC TGAGGCGGGC ACGCCGATGT 420
CCGTCATCGG CGAAGCGTCC AGCTGCGGTC AGGGAGGCCT GCTTCGAGGC ATACCGCTGG 480
CGAGAGCGGG GCGGCGTACA GGCGCCTTCG CTCACCGATA CGGGTTCCGC CACAGCGTGA 540
TTACCCGGTC TCCGTCGGCG GGATAGCTTT CGATCCGGTC TGCGCGGCCG CCGGAAATGC 600
TGCAGATAGC GATCGACCGC GCCGGTCGGT AAACGCCGCA CACGGCACTA TCAATGCGCA 660
CGGCGGGCGT TGATGCCAAA TTGACCGTCC CGACGGGGCT TTATCTGCGG CAAGATTTCA 720
TCCCCAGCCC GGTCGGTGGG CCGATAAATA CGCTGGTCAG CGCGACTCTT CCGGCTGAAT 780
TCGATGCTCT GGGCGCCCGC TCGACGCCGA GTATCTCGAG TGGGCCGCAA ACCCGGTCAA 840
ACGCTGTTAC TGTGGCGTTA CCACAGGTGA ATTTGCGGTG CCAACTGGTG AACACTTGCG 900
AACGGGTGGC ATCGAAATCA ACTTGTTGCG TTGCAGTGAT CTACTCTCTT GCAGAGAGCC 960
GTTGCTGGGA TTAATTGGGA GAGGAAGACA GCATGTCGTT CGTGACCACA CAGCCGGAAG 1020
CCCTGGCAGC TGCGGCGGCG AACCTACAGG GTATTGGCAC GACAATGAAC GCCCAGAACG 1080
CGGCCGCGGC TGCTCCAACC ACCGGAGTAG TGCCCGCAGC CGCCGATGAA GTATCAGCGC 1140
TGACCGCGGC TCAGTTTGCT GCGCACGCGC AGATGTACCA AACGGTCAGC GCCCAGGCCG 1200
CGGCCATTCA CGAAATGTTC GTGAACACGC TGGTGGCCAG TTCTGGCTCA TACGCGGCCA 1260
CCGAGGCGGC CAACGCAGCC GCTGCCGGCT GAACGGGCTC GCACGAACCT GCTGAAGGAG 1320
AGGGGGAACA TCCGGAGTTC TCGGGTCAGG GGTTGCGCCA GCGCCCAGCC GATTCAGCTA 1380
TCGGCGTCCA TAACAGCAGA CGATCTAGGC ATTCAGTACT AAGGAGACAG GCAACATGGC 1440
CTCACGTTTT ATGACGGATC CGCATGCGAT GCGGGACATG GCGGGCCGTT TTGAGGTGCA 1500
CGCCCAGACG GTGGAGGACG AGGCTCGCCG GATGTGGGCG TCCGCGCAAA ACATTTCCGG 1560
TGCGGGCTGG AGTGGCATGG CCGAGGCGAC CTCGCTAGAC ACCATGACCT AGATGAATCA 1620
GGCGTTTCGC AACATCGTGA ACATGCTGCA CGGGGTGCGT GACGGGCTGG TTCGCGACGC 1680
CAACAACTAC GAACAGCAAG AGCAGGCCTC CCAGCAGATC CTGAGCAGCT AGCGCCGAAA 1740
GCCACAGCTG CGTACGCTTT CTCACATTAG GAGAACACCA ATATGACGAT TAATTACCAG 1800
TTCGGGGACG TCGACGCTCA TGGCGCCATG ATCCGCGCTC AGGCGGCGTC GCTTGAGGCG 1860
GAGCATCAGG CCATCGTTCG TGATGTGTTG GCCGCGGGTG ACTTTTGGGG CGGCGCCGGT 1920
TCGGTGGCTT GCCAGGAGTT CATTACCCAG TTGGGCCGTA ACTTCCAGGT GATCTACGAG 1980
CAGGCCAACG CCCACGGGCA GAAGGTGCAG GCTGCCGGCA ACAACATGGC GCAAACCGAC 2040
AGCGCCGTCG GCTCCAGCTG GGCCTAAAAC TGAACTTCAG TCGCGGCAGC ACACCAACCA 2100
GCCGGTGTGC TGCTGTGTCC TGCAGTTAAC TAGCACTCGA CCGCTGAGGT AGCGATGGAT 2160
CAACAGAGTA CCCGCACCGA CATCACCGTC AACGTCGACG GCTTCTGGAT GCTTCAGGCG 2220
CTACTGGATA TCCGCCACGT TGCGCCTGAG TTACGTTGCC GGCCGTACGT CTCCACCGAT 2280
TCCAATGACT GGCTAAACGA GCACCCGGGG ATGGCGGTCA TGCGCGAGCA GGGCATTGTC 2340
GTCAACGACG CGGTCAACGA ACAGGTCGCT GCCCGGATGA AGGTGCTTGC CGCACCTGAT 2400
CTTGAAGTCG TCGCCCTGCT GTCACGCGGC AAGTTGCTGT ACGGGGTCAT AGACGACGAG 2460
AACCAGCCGC CGGGTTCGCG TGACATCCCT GACAATGAGT TCCGGGTGGT GTTGGCCCGG 2520
CGAGGCCAGC ACTGGGTGTC GGCGGTACGG GTTGGCAATG ACATCACCGT CGATGACGTG 2580
ACGGTCTCGG ATAGCGCCTC GATCGCCGCA CTGGTAATGG ACGGTCTGGA GTCGATTCAC 2640
CACGCCGACC CAGCCGCGAT CAACGCGGTC AACGTGCCAA TGGAGGAGAT CTCGTGCCGA 2700
ATTCGGCACG AGGCACGAGG CGGTGTCGGT GACGACGGGA TCGATCACGA TCATCGACCG 2760
GCCGGGATCC TTGGCGATCT CGTTGAGCAC GACCCGGGCC CGCGGGAAGC TCTGCGACAT 2820
CCATGGGTTC TTCCCG 2836

(2) INFORMATION FOR SEQ ID NO:27: MTI (MTB9.9A)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1               5                  10                  15
Ile Arg Ala Leu Ala Gly Leu Leu Glu Ala Glu His Gln Ala Ile Ile
            20                  25                  30
Ser Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
        35                  40                  45
Ala Cys Gln Gly Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
    50                  55                  60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65                  70                  75                  80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:28: HTCC#1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
CAGGCATGAG CAGAGCGTTC ATCATCGATC CAACGATCAG TGCCATTGAC GGCTTGTACG     60
ACCTTCTGGG GATTGGAATA CCCAACCAAG GGGGTATCCT TTACTCCTCA CTAGAGTACT    120
TCGAAAAAGC CCTGGAGGAG CTGGCAGCAG CGTTTCCGGG TGATGGCTGG TTAGGTTCGG    180
CCGCGGACAA ATACGCCGGC AAAAACCGCA ACCACGTGAA TTTTTTCCAG GAACTGGCAG    240
ACCTCGATCG TCAGCTCATC AGCCTGATCC ACGACCAGGC CAACGCGGTC CAGACGACCC    300
GCGACATCCT GGAGGGCGCC AAGAAAGGTC TCGAGTTCGT GCGCCCGGTG GCTGTGGACC    360
TGACCTACAT CCCGGTCGTC GGGCACGCCC TATCGGCCGC CTTCCAGGCG CCGTTTTGCG    420
CGGGCGCGAT GGCCGTAGTG GGCGGCGCGC TTGCCTACTT GGTCGTGAAA ACGCTGATCA    480
ACGCGACTCA ACTCCTCAAA TTGCTTGCCA AATTGGCGGA GTTGGTCGCG GCCGCCATTG    540
CGGACATCAT TTCGGATGTG GCGGACATCA TCAAGGGCAC CCTCGGAGAA GTGTGGGAGT    600
TCATCACAAA CGCGCTCAAC GGCCTGAAAG AGCTTTGGGA CAAGCTCACG GGGTGGGTGA    660
CCGGACTGTT CTCTCGAGGG TGGTCGAACC TGGAGTCCTT CTTTGCGGGC GTCCCCGGCT    720
TGACCGGCGC GACCAGCGGC TTGTCGCAAG TGACTGGCTT GTTCGGTGCG GCCGGTCTGT    780
CCGCATCGTC GGGCTTGGCT CACGCGGATA GCCTGGCGAG CTCAGCCAGC TTGCCCGCCC    840
TGGCCGGCAT TGGGGGCGGG TCCGGTTTTG GGGGCTTGCC GAGCCTGGCT CAGGTCCATG    900
CCGCCTCAAC TCGGCAGGCG CTACGGCCCC GAGCTGATGG CCCGGTCGGC GCCGCTGCCG    960
AGCAGGTCGG CGGGCAGTCG CAGCTGGTCT CCGCGCAGGG TTCCCAAGGT ATGGGCGGAC   1020
CCGTAGGCAT GGGCGGCATG CACCCCTCTT CGGGGGCGTC GAAAGGGACG ACGACGAAGA   1080
AGTACTCGGA AGGCGCGGCG GCGGGCACTG AAGACGCCGA GCGCGCGCCA GTCGAAGCTG   1140
ACGCGGGCGG TGGGCAAAAG GTGCTGGTAC GAAACGTCGT CTAACGGCAT GGCGAGCCAA   1200
```

(2) INFORMATION FOR SEQ ID NO:29: HTCC#1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 392 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Met Ser Arg Ala Phe Ile Ile Asp Pro Thr Ile Ser Ala Ile Asp Gly
 1               5                  10                  15
Leu Tyr Asp Leu Leu Gly Ile Gly Ile Pro Asn Gln Gly Gly Ile Leu
            20                  25                  30
Tyr Ser Ser Leu Glu Tyr Phe Glu Lys Ala Leu Glu Glu Leu Ala Ala
        35                  40                  45
Ala Phe Pro Gly Asp Gly Trp Leu Gly Ser Ala Ala Asp Lys Tyr Ala
    50                  55                  60
Gly Lys Asn Arg Asn His Val Asn Phe Phe Gln Glu Leu Ala Asp Leu
65                  70                  75                  80
Asp Arg Gln Leu Ile Ser Leu Ile His Asp Gln Ala Asn Ala Val Gln
                85                  90                  95
Thr Thr Arg Asp Ile Leu Glu Gly Ala Lys Lys Gly Leu Glu Phe Val
            100                 105                 110
Arg Pro Val Ala Val Asp Leu Thr Tyr Ile Pro Val Val Gly His Ala
        115                 120                 125
Leu Ser Ala Ala Phe Gln Ala Pro Phe Cys Ala Gly Ala Met Ala Val
    130                 135                 140
Val Gly Gly Ala Leu Ala Tyr Leu Val Val Lys Thr Leu Ile Asn Ala
145                 150                 155                 160
Thr Gln Leu Leu Lys Leu Leu Ala Lys Leu Ala Glu Leu Val Ala Ala
                165                 170                 175
Ala Ile Ala Asp Ile Ile Ser Asp Val Ala Asp Ile Ile Lys Gly Thr
            180                 185                 190
Leu Gly Glu Val Trp Glu Phe Ile Thr Asn Ala Leu Asn Gly Leu Lys
        195                 200                 205
Glu Leu Trp Asp Lys Leu Thr Gly Trp Val Thr Gly Leu Phe Ser Arg
    210                 215                 220
Gly Trp Ser Asn Leu Glu Ser Phe Phe Ala Gly Val Pro Gly Leu Thr
225                 230                 235                 240
Gly Ala Thr Ser Gly Leu Ser Gln Val Thr Gly Leu Phe Gly Ala Ala
                245                 250                 255
Gly Leu Ser Ala Ser Ser Gly Leu Ala His Ala Asp Ser Leu Ala Ser
            260                 265                 270
Ser Ala Ser Leu Pro Ala Leu Ala Gly Ile Gly Gly Gly Ser Gly Phe
        275                 280                 285
Gly Gly Leu Pro Ser Leu Ala Gln Val His Ala Ala Ser Thr Arg Gln
    290                 295                 300
Ala Leu Arg Pro Arg Ala Asp Gly Pro Val Gly Ala Ala Ala Glu Gln
305                 310                 315                 320
Val Gly Gly Gln Ser Gln Leu Val Ser Ala Gln Gly Ser Gln Gly Met
                325                 330                 335
Gly Gly Pro Val Gly Met Gly Gly Met His Pro Ser Ser Gly Ala Ser
            340                 345                 350
Lys Gly Thr Thr Thr Lys Lys Tyr Ser Glu Gly Ala Ala Ala Gly Thr
        355                 360                 365
Glu Asp Ala Glu Arg Ala Pro Val Glu Ala Asp Ala Gly Gly Gly Gln
    370                 375                 380
Lys Val Leu Val Arg Asn Val Val
385                 390
```

(2) INFORMATION FOR SEQ ID NO:30: MTCC#2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1441 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
GAGGTTGCTG GCAATGGATT TCGGGCTTTT ACCTCCGGAA GTGAATTCAA GCCGAATGTA     60
TTCCGGTCCG GGGCCGGAGT CGATGCTAGC CGCCGCGGCC GCCTGGGACG GTGTGGCCGC    120
GGAGTTGACT TCCGCCGCGG TCTCGTATGG ATCGGTGGTG TCGACGCTGA TCGTTGAGCC    180
GTGGATGGGG CCGGCGGCGG CCGCGATGGC GGCCGCGGCA ACGCCGTATG TGGGGTGGCT    240
```

```
GGCCGCCACG GCGGCGCTGG CGAAGGAGAC GGCCACACAG GCGAGGGCAG CGGCGGAAGC    300
GTTTGGGACG GCGTTCGCGA TGACGGTGCC ACCATCCCTC GTCGCGGCCA ACCGCAGCCG    360
GTTGATGTCG CTGGTCGCGG CGAACATTCT GGGGCAAAAC AGTGCGGCGA TCGCGGCTAC    420
CCAGGCCGAG TATGCCGAAA TGTGGGCCCA AGACGCTGCC GTGATGTACA GCTATGAGGG    480
GGCATCTGCG GCCGCGTCGG CGTTGCCGCC GTTCACTCCA CCCGTGCAAG GCACCGGCCC    540
GGCCGGGCCC GCGGCCGCAG CCGCGGCGAC CCAAGCCGCC GGTGCGGGCG CCGTTGCGGA    600
TGCACAGGCG ACACTGGCCC AGCTGCCCCC GGGGATCCTG AGCGACATTC TGTCCGCATT    660
GGCCGCCAAC GCTGATCCGC TGACATCGGG ACTGTTGGGG ATCGCGTCGA CCCTCAACCC    720
GCAAGTCGGA TCCGCTCAGC CGATAGTGAT CCCCACCCCG ATAGGGGAAT TGGACGTGAT    780
CGCGCTCTAC ATTGCATCCA TCGCGACCGG CAGCATTGCG CTCGCGATCA CGAACACGGC    840
CAGACCCTGG CACATCGGCC TATACGGGAA CGCCGGCGGG CTGGGACCGA CGCAGGGCCA    900
TCCACTGAGT TCGGCGACCG ACGAGCCGGA GCCGCACTGG GGCCCCTTCG GGGGCGCGGC    960
GCCGGTGTCC GCGGGCGTCG GCCACGCAGC ATTAGTCGGA GCGTTGTCGG TGCCGCACAG   1020
CTGGACCACG GCCGCCCCGG AGATCCAGCT CGCCGTTCAG GCAACACCCA CCTTCAGCTC   1080
CAGCGCCGGC GCCGACCCGA CGGCCCTAAA CGGGATGCCG GCAGGCCTGC TCAGCGGGAT   1140
GGCTTTGGCG AGCCTGGCCG CACGCGGCAC GACGGGCGGT GGCGGCACCC GTAGCGGCAC   1200
CAGCACTGAC GGCCAAGAGG ACGGCCGCAA ACCCCCGGTA GTTGTGATTA GAGAGCAGCC   1260
GCCGCCCGGA AACCCCCCGC GGTAAAAGTC CGGCAACCGT TCGTCGCCGC GCGGAAAATG   1320
CCTGGTGAGC GTGGCTATCC GACGGGCCGT TCACACCGCT TGTAGTAGCG TACGGCTATG   1380
GACGACGGTG TCTGGATTCT CGGCGGCTAT CAGAGCGATT TTGCTCGCAA CCTCAGCAAA   1440
G                                                                   1441
```

(2) INFORMATION FOR SEQ ID NO:31: MTCC#2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Asp Phe Gly Leu Leu Pro Pro Glu Val Asn Ser Ser Arg Met Tyr
 1               5                  10                  15
Ser Gly Pro Gly Pro Glu Ser Met Leu Ala Ala Ala Ala Ala Trp Asp
            20                  25                  30
Gly Val Ala Ala Glu Leu Thr Ser Ala Ala Val Ser Tyr Gly Ser Val
        35                  40                  45
Val Ser Thr Leu Ile Val Glu Pro Trp Met Gly Pro Ala Ala Ala Ala
    50                  55                  60
Met Ala Ala Ala Ala Thr Pro Tyr Val Gly Trp Leu Ala Ala Thr Ala
65                  70                  75                  80
Ala Leu Ala Lys Glu Thr Ala Thr Gln Ala Arg Ala Ala Ala Glu Ala
                85                  90                  95
Phe Gly Thr Ala Phe Ala Met Thr Val Pro Pro Ser Leu Val Ala Ala
            100                 105                 110
Asn Arg Ser Arg Leu Met Ser Leu Val Ala Ala Asn Ile Leu Gly Gln
        115                 120                 125
Asn Ser Ala Ala Ile Ala Ala Thr Gln Ala Glu Tyr Ala Glu Met Trp
    130                 135                 140
Ala Gln Asp Ala Ala Val Met Tyr Ser Tyr Glu Gly Ala Ser Ala Ala
145                 150                 155                 160
Ala Ser Ala Leu Pro Pro Phe Thr Pro Pro Val Gln Gly Thr Gly Pro
                165                 170                 175
Ala Gly Pro Ala Ala Ala Ala Ala Ala Thr Gln Ala Ala Gly Ala Gly
            180                 185                 190
Ala Val Ala Asp Ala Gln Ala Thr Leu Ala Gln Leu Pro Pro Gly Ile
        195                 200                 205
Leu Ser Asp Ile Leu Ser Ala Leu Ala Ala Asn Ala Asp Pro Leu Thr
    210                 215                 220
Ser Gly Leu Leu Gly Ile Ala Ser Thr Leu Asn Pro Gln Val Gly Ser
225                 230                 235                 240
Ala Gln Pro Ile Val Ile Pro Thr Pro Ile Gly Glu Leu Asp Val Ile
                245                 250                 255
Ala Leu Tyr Ile Ala Ser Ile Ala Thr Gly Ser Ile Ala Leu Ala Ile
```

```
            260                 265                 270
Thr Asn Thr Ala Arg Pro Trp His Ile Gly Leu Tyr Gly Asn Ala Gly
        275                 280                 285
Gly Leu Gly Pro Thr Gln Gly His Pro Leu Ser Ser Ala Thr Asp Glu
    290                 295                 300
Pro Glu Pro His Trp Gly Pro Phe Gly Gly Ala Ala Pro Val Ser Ala
305                 310                 315                 320
Gly Val Gly His Ala Ala Leu Val Gly Ala Leu Ser Val Pro His Ser
                325                 330                 335
Trp Thr Thr Ala Ala Pro Glu Ile Gln Leu Ala Val Gln Ala Thr Pro
            340                 345                 350
Thr Phe Ser Ser Ser Ala Gly Ala Asp Pro Thr Ala Leu Asn Gly Met
        355                 360                 365
Pro Ala Gly Leu Leu Ser Gly Met Ala Leu Ala Ser Leu Ala Ala Arg
    370                 375                 380
Gly Thr Thr Gly Gly Gly Gly Thr Arg Ser Gly Thr Ser Thr Asp Gly
385                 390                 395                 400
Gln Glu Asp Gly Arg Lys Pro Pro Val Val Val Ile Arg Glu Gln Pro
                405                 410                 415
Pro Pro Gly Asn Pro Pro Arg
            420
```

(2) INFORMATION FOR SEQ ID NO:32: ESAT-6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
ATGACAGAGC AGCAGTGGAA TTTCGCGGGT ATCGAGGCCG CGGCAAGCGC AATCCAGGGA      60
AATGTCACGT CCATTCATTC CTCCTTGAC GAGGGGAAGC AGTCCCTGAC CAAGCTCGCA      120
GCGGCCTGGG GCGGTAGCGG TTCGGAAGCG TACC                                  154
```

(2) INFORMATION FOR SEQ ID NO:33: ESAT-6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Met Thr Glu Gln Gln Trp Asn Phe Ala Gly Ile Glu Ala Ala Ala Ser
1               5                   10                  15
Ala Ile Gln Gly Asn Val Thr Ser Ile His Ser Leu Leu Asp Glu Gly
            20                  25                  30
Lys Gln Ser Leu Thr Lys Leu Ala Ala Ala Trp Gly Gly Ser Gly Ser
        35                  40                  45
Glu Ala Tyr
    50
```

(2) INFORMATION FOR SEQ ID NO:34: Tb38-1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
CGGCACGAGA GACCGATGCC GCTACCCTCG CGCAGGAGGC AGGTAATTTC GAGCGGATCT      60
CCGGCGACCT GAAAACCCAG ATCGACCAGG TGGAGTCGAC GGCAGGTTCG TTGCAGGGCC     120
AGTGGCGCGG CGCGGCGGGG ACGGCCGCCC AGGCCGCGGT GGTGCGCTTC CAAGAAGCAG     180
CCAATAAGCA GAAGCAGGAA CTCGACGAGA TCTCGACGAA TATTCGTCAG GCCGGCGTCC     240
AATACTCGAG GGCCGACGAG GAGCAGCAGC AGGCGCTGTC CTCGCAAATG GGCTTCTGAC     300
CCGCTAATAC GAAAAGAAAC GGAGCAA                                         327
```

(2) INFORMATION FOR SEQ ID NO:35: Tb38-1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Thr Asp Ala Ala Thr Leu Ala Gln Glu Ala Gly Asn Phe Glu Arg Ile
1               5                  10                  15
Ser Gly Asp Leu Lys Thr Gln Ile Asp Gln Val Glu Ser Thr Ala Gly
            20                  25                  30
Ser Leu Gln Gly Gln Trp Arg Gly Ala Ala Gly Thr Ala Ala Gln Ala
        35                  40                  45
Ala Val Val Arg Phe Gln Glu Ala Ala Asn Lys Gln Lys Gln Glu Leu
    50                  55                  60
Asp Glu Ile Ser Thr Asn Ile Arg Gln Ala Gly Val Gln Tyr Ser Arg
65                  70                  75                  80
Ala Asp Glu Glu Gln Gln Gln Ala Leu Ser Ser Gln Met Gly Phe
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:36: TbRa3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 542 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
GAATTCGGCA CGAGAGGTGA TCGACATCAT CGGGACCAGC CCCACATCCT GGGAACAGGC      60
GGCGGCGGAG GCGGTCCAGC GGGCGCGGGA TAGCGTCGAT GACATCCGCG TCGCTCGGGT     120
CATTGAGCAG GACATGGCCG TGGACAGCGC CGGCAAGATC ACCTACCGCA TCAAGCTCGA     180
AGTGTCGTTC AAGATGAGGC CGGCGCAACC GCGCTAGCAC GGGCCGGCGA GCAAGACGCA     240
AAATCGCACG GTTTGCGGTT GATTCGTGCG ATTTTGTGTC TGCTCGCCGA GGCCTACCAG     300
GCGCGGCCCA GGTCCGCGTG CTGCCGTATC CAGGCGTGCA TCGCGATTCC GGCGGCCACG     360
CCGGAGTTAA TGCTTCGCGT CGACCCGAAC TGGGCGATCC GCCGGNGAGC TGATCGATGA     420
CCGTGGCCAG CCCGTCGATG CCCGAGTTGC CCGAGGAAAC GTGCTGCCAG GCCGGTAGGA     480
AGCGTCCGTA GGCGGCGGTG CTGACCGGCT CTGCCTGCGC CCTCAGTGCG GCCAGCGAGC     540
GG                                                                    542
```

(2) INFORMATION FOR SEQ ID NO:37: TbRa3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Val Ile Asp Ile Ile Gly Thr Ser Pro Thr Ser Trp Glu Gln Ala Ala
1               5                  10                  15
Ala Glu Ala Val Gln Arg Ala Arg Asp Ser Val Asp Asp Ile Arg Val
            20                  25                  30
```

Ala Arg Val Ile Glu Gln Asp Met Ala Val Asp Ser Ala Gly Lys Ile
35 40 45
Thr Tyr Arg Ile Lys Leu Glu Val Ser Phe Lys Met Arg Pro Ala Gln
50 55 60
Pro Arg
65

(2) INFORMATION FOR SEQ ID NO:38: 38 kD

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1993 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120
GCGGAAATTG AAGAGCACAG AAAGGTATGG CGTGAAAATT CGTTTGCATA CGCTGTTGGC 180
CGTGTTGACC GCTGCGCCGC TGCTGCTAGC AGCGGCGGGC TGTGGCTCGA AACCACCGAG 240
CGGTTCGCCT GAAACGGGCG CCGGCGCCGG TACTGTCGCG ACTACCCCCG CGTCGTCGCC 300
GGTGACGTTG GCGGAGACCG GTAGCACGCT GCTCTACCCG CTGTTCAACC TGTGGGGTCC 360
GGCCTTTCAC GAGAGGTATC CGAACGTCAC GATCACCGCT CAGGGCACCG GTTCTGGTGC 420
CGGGATCGCG CAGGCCGCCG CCGGGACGGT CAACATTGGG GCCTCCGACG CCTATCTGTC 480
GGAAGGTGAT ATGGCCGCGC ACAAGGGGCT GATGAACATC GCGCTAGCCA TCTCCGCTCA 540
GCAGGTCAAC TACAACCTGC CCGGAGTGAG CGAGCACCTC AAGCTGAACG GAAAAGTCCT 600
GGCGGCCATG TACCAGGGCA CCATCAAAAC CTGGGACGAC CCGCAGATCG CTGCGCTCAA 660
CCCCGGCGTG AACCTGCCCG GCACCGCGGT AGTTCCGCTG CACCGCTCCG ACGGGTCCGG 720
TGACACCTTC TTGTTCACCC AGTACCTGTC CAAGCAAGAT CCCGAGGGCT GGGGCAAGTC 780
GCCCGGCTTC GGCACCACCG TCGACTTCCC GGCGGTGCCG GGTGCGCTGG GTGAGAACGG 840
CAACGGCGGC ATGGTGACCG GTTGCGCCGA GACACCGGGC TGCGTGGCCT ATATCGGCAT 900
CAGCTTCCTC GACCAGGCCA GTCAACGGGG ACTCGGCGAG GCCCAACTAG GCAATAGCTC 960
TGGCAATTTC TTGTTGCCCG ACGCGCAAAG CATTCAGGCC GCGGCGGCTG GCTTCGCATC 1020
GAAAACCCCG GCGAACCAGG CGATTTCGAT GATCGACGGG CCCGCCCCGG ACGGCTACCC 1080
GATCATCAAC TACGAGTACG CCATCGTCAA CAACCGGCAA AAGGACGCCG CCACCGCGCA 1140
GACCTTGCAG GCATTTCTGC ACTGGGCGAT CACCGACGGC AACAAGGCCT CGTTCCTCGA 1200
CCAGGTTCAT TTCCAGCCGC TGCCGCCCGC GGTGGTGAAG TTGTCTGACG CGTTGATCGC 1260
GACGATTTCC AGCTAGCCTC GTTGACCACC ACGCGACAGC AACCTCCGTC GGGCCATCGG 1320
GCTGCTTTGC GGAGCATGCT GGCCCGTGCC GGTGAAGTCG GCCGCGCTGG CCCGGCCATC 1380
CGGTGGTTGG GTGGGATAGG TGCGGTGATC CCGCTGCTTG CGCTGGTCTT GGTGCTGGTG 1440
GTGCTGGTCA TCGAGGCGAT GGGTGCGATC AGGCTCAACG GGTTGCATTT CTTCACCGCC 1500

ACCGAATGGA ATCCAGGCAA CACCTACGGC GAAACCGTTG TCACCGACGC GTCGCCCATC 1560

CGGTCGGCGC CTACTACGGG GCGTTGCCGC TGATCGTCGG GACGCTGGCG ACCTCGGCAA 1620

TCGCCCTGAT CATCGCGGTG CCGGTCTCTG TAGGAGCGGC GCTGGTGATC GTGGAACGGC 1680

TGCCGAAACG GTTGGCCGAG GCTGTGGGAA TAGTCCTGGA ATTGCTCGCC GGAATCCCCA 1740

GCGTGGTCGT CGGTTTGTGG GGGGCAATGA CGTTCGGGCC GTTCATCGCT CATCACATCG 1800

CTCCGGTGAT CGCTCACAAC GCTCCCGATG TGCCGGTGCT GAACTACTTG CGCGGCGACC 1860

CGGGCAACGG GGAGGGCATG TTGGTGTCCG GTCTGGTGTT GGCGGTGATG GTCGTTCCCA 1920

TTATCGCCAC CACCACTCAT GACCTGTTCC GGCAGGTGCC GGTGTTGCCC CGGGAGGGCG 1980

CGATCGGGAA TTC 1993

(2) INFORMATION FOR SEQ ID NO:39: 38 kD

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Lys Ile Arg Leu His Thr Leu Leu Ala Val Leu Thr Ala Ala Pro
1 5 10 15

Leu Leu Leu Ala Ala Ala Gly Cys Gly Ser Lys Pro Pro Ser Gly Ser
20 25 30

Pro Glu Thr Gly Ala Gly Ala Gly Thr Val Ala Thr Thr Pro Ala Ser
35 40 45

Ser Pro Val Thr Leu Ala Glu Thr Gly Ser Thr Leu Leu Tyr Pro Leu
50 55 60

Phe Asn Leu Trp Gly Pro Ala Phe His Glu Arg Tyr Pro Asn Val Thr
65 70 75 80

Ile Thr Ala Gln Gly Thr Gly Ser Gly Ala Gly Ile Ala Gln Ala Ala
85 90 95

Ala Gly Thr Val Asn Ile Gly Ala Ser Asp Ala Tyr Leu Ser Glu Gly
100 105 110

Asp Met Ala Ala His Lys Gly Leu Met Asn Ile Ala Leu Ala Ile Ser
115 120 125

Ala Gln Gln Val Asn Tyr Asn Leu Pro Gly Val Ser Glu His Leu Lys
130 135 140

Leu Asn Gly Lys Val Leu Ala Ala Met Tyr Gln Gly Thr Ile Lys Thr
145 150 155 160

Trp Asp Asp Pro Gln Ile Ala Ala Leu Asn Pro Gly Val Asn Leu Pro
165 170 175

Gly Thr Ala Val Val Pro Leu His Arg Ser Asp Gly Ser Gly Asp Thr
180 185 190

Phe Leu Phe Thr Gln Tyr Leu Ser Lys Gln Asp Pro Glu Gly Trp Gly
195 200 205

```
Lys Ser Pro Gly Phe Gly Thr Thr Val Asp Phe Pro Ala Val Pro Gly
    210                 215                 220

Ala Leu Gly Glu Asn Gly Asn Gly Gly Met Val Thr Gly Cys Ala Glu
225                 230                 235                 240

Thr Pro Gly Cys Val Ala Tyr Ile Gly Ile Ser Phe Leu Asp Gln Ala
                245                 250                 255

Ser Gln Arg Gly Leu Gly Glu Ala Gln Leu Gly Asn Ser Ser Gly Asn
            260                 265                 270

Phe Leu Leu Pro Asp Ala Gln Ser Ile Gln Ala Ala Ala Ala Gly Phe
        275                 280                 285

Ala Ser Lys Thr Pro Ala Asn Gln Ala Ile Ser Met Ile Asp Gly Pro
    290                 295                 300

Ala Pro Asp Gly Tyr Pro Ile Ile Asn Tyr Glu Tyr Ala Ile Val Asn
305                 310                 315                 320

Asn Arg Gln Lys Asp Ala Ala Thr Ala Gln Thr Leu Gln Ala Phe Leu
                325                 330                 335

His Trp Ala Ile Thr Asp Gly Asn Lys Ala Ser Phe Leu Asp Gln Val
            340                 345                 350

His Phe Gln Pro Leu Pro Pro Ala Val Val Lys Leu Ser Asp Ala Leu
        355                 360                 365

Ile Ala Thr Ile Ser Ser
    370
```

(2) INFORMATION FOR SEQ ID NO:40: DPEP

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 999 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
ATGCATCACC ATCACCATCA CATGCATCAG GTGGACCCCA ACTTGACACG TCGCAAGGGA     60
CGATTGGCGG CACTGGCTAT CGCGGCGATG GCCAGCGCCA GCCTGGTGAC CGTTGCGGTG    120
CCCGCGACCG CCAACGCCGA TCCGGAGCCA GCGCCCCCGG TACCCACAAC GGCCGCCTCG    180
CCGCCGTCGA CCGCTGCAGC GCCACCCGCA CCGGCGACAC CTGTTGCCCC CCCACCACCG    240
GCCGCCGCCA ACACGCCGAA TGCCCAGCCG GGCGATCCCA ACGCAGCACC TCCGCCGGCC    300
GACCCGAACG CACCGCCGCC ACCTGTCATT GCCCCAAACG CACCCCAACC TGTCCGGATC    360
GACAACCCGG TTGGAGGATT CAGCTTCGCG CTGCCTGCTG GCTGGGTGGA GTCTGACGCC    420
GCCCACTTCG ACTACGGTTC AGCACTCCTC AGCAAAACCA CCGGGGACCC GCCATTTCCC    480
GGACAGCCGC CGCCGGTGGC CAATGACACC CGTATCGTGC TCGGCCGGCT AGACCAAAAG    540
CTTTACGCCA GCGCCGAAGC CACCGACTCC AAGGCCGCGG CCCGGTTGGG CTCGGACATG    600
GGTGAGTTCT ATATGCCCTA CCCGGGCACC CGGATCAACC AGGAAACCGT CTCGCTCGAC    660
GCCAACGGGG TGTCTGGAAG CGCGTCGTAT TACGAAGTCA AGTTCAGCGA TCCGAGTAAG    720
CCGAACGGCC AGATCTGGAC GGGCGTAATC GGCTCGCCCG CGGCGAACGC ACCGGACGCC    780
GGGCCCCCTC AGCGCTGGTT TGTGGTATGG CTCGGGACCG CCAACAACCC GGTGGACAAG    840
GGCGCGGCCA AGGCGCTGGC CGAATCGATC CGGCCTTTGG TCGCCCCGCC GCCGGCGCCG    900
GCACCGGCTC CTGCAGAGCC CGCTCCGGCG CCGGCGCCGG CCGGGGAAGT CGCTCCTACC    960
CCGACGACAC CGACACCGCA GCGGACCTTA CCGGCCTGA                           999
```

(2) INFORMATION FOR SEQ ID NO:41: DPEP

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 332 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Met His His His His His His Met His Gln Val Asp Pro Asn Leu Thr
1               5                  10                  15
Arg Arg Lys Gly Arg Leu Ala Ala Leu Ala Ile Ala Ala Met Ala Ser
            20                  25                  30
Ala Ser Leu Val Thr Val Ala Val Pro Ala Thr Ala Asn Ala Asp Pro
        35                  40                  45
Glu Pro Ala Pro Pro Val Pro Thr Thr Ala Ala Ser Pro Pro Ser Thr
    50                  55                  60
Ala Ala Ala Pro Pro Ala Pro Ala Thr Pro Val Ala Pro Pro Pro Pro
65                  70                  75                  80
Ala Ala Ala Asn Thr Pro Asn Ala Gln Pro Gly Asp Pro Asn Ala Ala
                85                  90                  95
Pro Pro Pro Ala Asp Pro Asn Ala Pro Pro Pro Pro Val Ile Ala Pro
            100                 105                 110
Asn Ala Pro Gln Pro Val Arg Ile Asp Asn Pro Val Gly Gly Phe Ser
        115                 120                 125
Phe Ala Leu Pro Ala Gly Trp Val Glu Ser Asp Ala Ala His Phe Asp
    130                 135                 140
Tyr Gly Ser Ala Leu Leu Ser Lys Thr Thr Gly Asp Pro Pro Phe Pro
145                 150                 155                 160
Gly Gln Pro Pro Pro Val Ala Asn Asp Thr Arg Ile Val Leu Gly Arg
                165                 170                 175
Leu Asp Gln Lys Leu Tyr Ala Ser Ala Glu Ala Thr Asp Ser Lys Ala
            180                 185                 190
Ala Ala Arg Leu Gly Ser Asp Met Gly Glu Phe Tyr Met Pro Tyr Pro
        195                 200                 205
Gly Thr Arg Ile Asn Gln Glu Thr Val Ser Leu Asp Ala Asn Gly Val
    210                 215                 220
Ser Gly Ser Ala Ser Tyr Tyr Glu Val Lys Phe Ser Asp Pro Ser Lys
225                 230                 235                 240
Pro Asn Gly Gln Ile Trp Thr Gly Val Ile Gly Ser Pro Ala Ala Asn
                245                 250                 255
Ala Pro Asp Ala Gly Pro Pro Gln Arg Trp Phe Val Val Trp Leu Gly
            260                 265                 270
Thr Ala Asn Asn Pro Val Asp Lys Gly Ala Ala Lys Ala Leu Ala Glu
        275                 280                 285
Ser Ile Arg Pro Leu Val Ala Pro Pro Pro Ala Pro Ala Pro Ala Pro
    290                 295                 300
Ala Glu Pro Ala Pro Ala Pro Ala Pro Ala Gly Glu Val Ala Pro Thr
305                 310                 315                 320
Pro Thr Thr Pro Thr Pro Gln Arg Thr Leu Pro Ala
                325                 330
```

(2) INFORMATION FOR SEQ ID NO:42: TbH4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 702 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
CGGCACGAGG ATCGGTACCC CGCGGCATCG GCAGCTGCCG ATTCGCCGGG TTTCCCCACC      60
CGAGGAAAGC CGCTACCAGA TGGCGCTGCC GAAGTAGGGC GATCCGTTCG CGATGCCGGC     120
ATGAACGGGC GGCATCAAAT TAGTGCAGGA ACCTTTCAGT TTAGCGACGA TAATGGCTAT     180
AGCACTAAGG AGGATGATCC GATATGACGC AGTCGCAGAC CGTGACGGTG GATCAGCAAG     240
AGATTTTGAA CAGGGCCAAC GAGGTGGAGG CCCCGATGGC GGACCCACCG ACTGATGTCC     300
CCATCACACC GTGCGAACTC ACGGNGGNTA AAAACGCCGC CCAACAGNTG GTNTTGTCCG     360
CCGACAACAT GCGGGAATAC CTGGCGGCCG GTGCCAAAGA GCGGCAGCGT CTGGCGACCT     420
CGCTGCGCAA CGCGGCCAAG GNGTATGGCG AGGTTGATGA GGAGGCTGCG ACCGCGCTGG     480
ACAACGACGG CGAAGGAACT GTGCAGGCAG AATCGGCCGG GGCCGTCGGA GGGGACAGTT     540
```

```
CGGCCGAACT AACCGATACG CCGAGGGTGG CCACGGCCGG TGAACCCAAC TTCATGGATC     600
TCAAAGAAGC GGCAAGGAAG CTCGAAACGG GCGACCAAGG CGCATCGCTC GCGCACTGNG     660
GGGATGGGTG GAACACTTNC ACCCTGACGC TGCAAGGCGA CG                        702
```

(2) INFORMATION FOR SEQ ID NO:43: TbH4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 286 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Gly Asp Ser Phe Trp Ala Ala Ala Asp Gln Met Ala Arg Gly Phe Val
1               5                  10                  15
Leu Gly Ala Thr Ala Gly Arg Thr Thr Leu Thr Gly Glu Gly Leu Gln
            20                  25                  30
His Ala Asp Gly His Ser Leu Leu Leu Asp Ala Thr Asn Pro Ala Val
        35                  40                  45
Val Ala Tyr Asp Pro Ala Phe Ala Tyr Glu Ile Gly Tyr Ile Xaa Glu
    50                  55                  60
Ser Gly Leu Ala Arg Met Cys Gly Glu Asn Pro Glu Asn Ile Phe Phe
65                  70                  75                  80
Tyr Ile Thr Val Tyr Asn Glu Pro Tyr Val Gln Pro Pro Glu Pro Glu
                85                  90                  95
Asn Phe Asp Pro Glu Gly Val Leu Gly Gly Ile Tyr Arg Tyr His Ala
            100                 105                 110
Ala Thr Glu Gln Arg Thr Asn Lys Xaa Gln Ile Leu Ala Ser Gly Val
        115                 120                 125
Ala Met Pro Ala Ala Leu Arg Ala Ala Gln Met Leu Ala Ala Glu Trp
    130                 135                 140
Asp Val Ala Ala Asp Val Trp Ser Val Thr Ser Trp Gly Glu Leu Asn
145                 150                 155                 160
Arg Asp Gly Val Val Ile Glu Thr Glu Lys Leu Arg His Pro Asp Arg
                165                 170                 175
Pro Ala Gly Val Pro Tyr Val Thr Arg Ala Leu Glu Asn Ala Arg Gly
            180                 185                 190
Pro Val Ile Ala Val Ser Asp Trp Met Arg Ala Val Pro Glu Gln Ile
        195                 200                 205
Arg Pro Trp Val Pro Gly Thr Tyr Leu Thr Leu Gly Thr Asp Gly Phe
    210                 215                 220
Gly Phe Ser Asp Thr Arg Pro Ala Gly Arg Arg Tyr Phe Asn Thr Asp
225                 230                 235                 240
Ala Glu Ser Gln Val Gly Arg Gly Phe Gly Arg Gly Trp Pro Gly Arg
                245                 250                 255
Arg Val Asn Ile Asp Pro Phe Gly Ala Gly Arg Gly Pro Pro Ala Gln
            260                 265                 270
Leu Pro Gly Phe Asp Glu Gly Gly Gly Leu Arg Pro Xaa Lys
        275                 280                 285
```

(2) INFORMATION FOR SEQ ID NO:44: DPPD

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
ATGAAGTTGA AGTTTGCTCG CCTGAGTACT GCGATACTGG GTTGTGCAGC GGCGCTTGTG      60
TTTCCTGCCT CGGTTGCCAG CGCAGATCCA CCTGACCCGC ATCAGCCGGA CATGACGAAA     120
GGCTATTGCC CGGGTGGCCG ATGGGGTTTT GGCGACTTGG CCGTGTGCGA CGGCGAGAAG     180
```

```
TACCCCGACG GCTCGTTTTG GCACCAGTGG ATGCAAACGT GGTTTACCGG CCCACAGTTT    240
TACTTCGATT GTGTCAGCGG CGGTGAGCCC CTCCCCGGCC CGCCGCCACC GGGTGGTTGC    300
GGTGGGGCAA TTCCGTCCGA GCAGCCCAAC GCTCCCTGA                        339
```

(2) INFORMATION FOR SEQ ID NO:45: DPPD

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
Met Lys Leu Lys Phe Ala Arg Leu Ser Thr Ala Ile Leu Gly Cys Ala
 1               5                  10                  15
Ala Ala Leu Val Phe Pro Ala Ser Val Ala Ser Ala Asp Pro Pro Asp
            20                  25                  30
Pro His Gln Pro Asp Met Thr Lys Gly Tyr Cys Pro Gly Gly Arg Trp
        35                  40                  45
Gly Phe Gly Asp Leu Ala Val Cys Asp Gly Glu Lys Tyr Pro Asp Gly
    50                  55                  60
Ser Phe Trp His Gln Trp Met Gln Thr Trp Phe Thr Gly Pro Gln Phe
65                  70                  75                  80
Tyr Phe Asp Cys Val Ser Gly Gly Glu Pro Leu Pro Gly Pro Pro Pro
                85                  90                  95
Pro Gly Gly Cys Gly Gly Ala Ile Pro Ser Glu Gln Pro Asn Ala Pro
            100                 105                 110
```

<210> SEQ ID NO:46
<211> 921
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:tri-fusion protein DPV-MTI-MSL (designated Mtb31f)
<222> (1)..(900)

```
cat atg cat cac cat cac cat cac gat ccc gtg gac gcg gtc att aac   48
His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
 1               5                  10                  15

acc acc tgc aat tac ggg cag gta gta gct gcg ctc aac gcg acg gat   96
Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
            20                  25                  30

ccg ggg gct gcc gca cag ttc aac gcc tca ccg gtg gcg cag tcc tat  144
Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
        35                  40                  45

ttg cgc aat ttc ctc gcc gca ccg cca cct cag cgc gct gcc atg gcc  192
Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
    50                  55                  60

gcg caa ttg caa gct gtg ccg ggg gcg gca cag tac atc ggc ctt gtc  240
Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
65                  70                  75                  80

gag tcg gtt gcc ggc tcc tgc aac aac tat gag ctc atg acg att aat  288
Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                85                  90                  95

tac cag ttc ggg gac gtc gac gct cat ggc gcc atg atc cgc gct cag  336
Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110
```

```
gcg gcg tcg ctt gag gcg gag cat cag gcc atc gtt cgt gat gtg ttg   384
Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

gcc gcg ggt gac ttt tgg ggc ggc gcc ggt tcg gtg gct tgc cag gag   432
Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140

ttc att acc cag ttg ggc cgt aac ttc cag gtg atc tac gag cag gcc   480
Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
145                 150                 155                 160

aac gcc cac ggg cag aag gtg cag gct gcc ggc aac aac atg gcg caa   528
Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
                165                 170                 175

acc gac agc gcc gtc ggc tcc agc tgg gcc act agt atg agc ctt ttg   576
Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
            180                 185                 190

gat gct cat atc cca cag ttg gtg gcc tcc cag tcg gcg ttt gcc gcc   624
Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
        195                 200                 205

aag gcg ggg ctg atg cgg cac acg atc ggt cag gcc gag cag gcg gcg   672
Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
    210                 215                 220

atg tcg gct cag gcg ttt cac cag ggg gag tcg tcg gcg gcg ttt cag   720
Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225                 230                 235                 240

gcc gcc cat gcc cgg ttt gtg gcg gcg gcc gcc aaa gtc aac acc ttg   768
Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys Val Asn Thr Leu
                245                 250                 255

ttg gat gtc gcg cag gcg aat ctg ggt gag gcc gcc ggt acc tat gtg   816
Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
            260                 265                 270

gcc gcc gat gct gcg gcc gcg tcg acc tat acc ggg ttc gat atc cat   864
Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile His
        275                 280                 285

cac act ggc ggc cgc tcg agc aga tcc ggc tgc taacaaagcc cgaaaggaag 917
His Thr Gly Gly Arg Ser Ser Arg Ser Gly Cys
    290                 295

ctga                                                              921
```

<210> SEQ ID NO:47
<211> 299
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:tri-fusion protein DPV-MTI-MSL (designated Mtb31f)

```
His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
  1               5                  10                  15

Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
             20                  25                  30

Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
         35                  40                  45
```

```
Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
     50                  55                  60

Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
 65                  70                  75                  80

Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                 85                  90                  95

Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110

Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140

Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
145                 150                 155                 160

Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
                165                 170                 175

Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
            180                 185                 190

Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
        195                 200                 205

Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
    210                 215                 220

Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225                 230                 235                 240

Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys Val Asn Thr Leu
                245                 250                 255

Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
            260                 265                 270

Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile His
        275                 280                 285

His Thr Gly Gly Arg Ser Ser Arg Ser Gly Cys
    290                 295
```

<210> SEQ ID NO:48
<211> 2168
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:tetra-fusion protein DPV-MTI-MSL-MTCC2 (designated Mtb71f)
<222> (1)..(2133)

```
cat atg cat cac cat cac cat cac gat ccc gtg gac gcg gtc att aac   48
His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
  1               5                  10                  15

acc acc tgc aat tac ggg cag gta gta gct gcg ctc aac gcg acg gat   96
Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
             20                  25                  30

ccg ggg gct gcc gca cag ttc aac gcc tca ccg gtg gcg cag tcc tat  144
Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
         35                  40                  45
```

```
ttg cgc aat ttc ctc gcc gca ccg cca cct cag cgc gct gcc atg gcc   192
Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
     50                  55                  60

gcg caa ttg caa gct gtg ccg ggg gcg gca cag tac atc ggc ctt gtc   240
Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
 65                  70                  75                  80

gag tcg gtt gcc ggc tcc tgc aac aac tat gag ctc atg acg att aat   288
Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                 85                  90                  95

tac cag ttc ggg gac gtc gac gct cat ggc gcc atg atc cgc gct cag   336
Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110

gcg gcg tcg ctt gag gcg gag cat cag gcc atc gtt cgt gat gtg ttg   384
Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

gcc gcg ggt gac ttt tgg ggc ggc gcc ggt tcg gtg gct tgc cag gag   432
Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140

ttc att acc cag ttg ggc cgt aac ttc cag gtg atc tac gag cag gcc   480
Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
145                 150                 155                 160

aac gcc cac ggg cag aag gtg cag gct gcc ggc aac aac atg gcg caa   528
Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
                165                 170                 175

acc gac agc gcc gtc ggc tcc agc tgg gcc act agt atg agc ctt ttg   576
Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
            180                 185                 190

gat gct cat atc cca cag ttg gtg gcc tcc cag tcg gcg ttt gcc gcc   624
Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
        195                 200                 205

aag gcg ggg ctg atg cgg cac acg atc ggt cag gcc gag cag gcg gcg   672
Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
    210                 215                 220

atg tcg gct cag gcg ttt cac cag ggg gag tcg tcg gcg gcg ttt cag   720
Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225                 230                 235                 240

gcc gcc cat gcc cgg ttt gtg gcg gcg gcc gcc aaa gtc aac acc ttg   768
Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys Val Asn Thr Leu
                245                 250                 255

ttg gat gtc gcg cag gcg aat ctg ggt gag gcc gcc ggt acc tat gtg   816
Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
            260                 265                 270

gcc gcc gat gct gcg gcc gcg tcg acc tat acc ggg ttc gat atc atg   864
Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile Met
        275                 280                 285

gat ttc ggg ctt tta cct ccg gaa gtg aat tca agc cga atg tat tcc   912
Asp Phe Gly Leu Leu Pro Pro Glu Val Asn Ser Ser Arg Met Tyr Ser
    290                 295                 300

ggt ccg ggg ccg gag tcg atg cta gcc gcc gcg gcc gcc tgg gac ggt   960
Gly Pro Gly Pro Glu Ser Met Leu Ala Ala Ala Ala Ala Trp Asp Gly
305                 310                 315                 320
```

```
gtg gcc gcg gag ttg act tcc gcc gcg gtc tcg tat gga tcg gtg gtg   1008
Val Ala Ala Glu Leu Thr Ser Ala Ala Val Ser Tyr Gly Ser Val Val
                325                 330                 335

tcg acg ctg atc gtt gag ccg tgg atg ggg ccg gcg gcg gcc gcg atg   1056
Ser Thr Leu Ile Val Glu Pro Trp Met Gly Pro Ala Ala Ala Ala Met
            340                 345                 350

gcg gcc gcg gca acg ccg tat gtg ggg tgg ctg gcc gcc acg gcg gcg   1104
Ala Ala Ala Ala Thr Pro Tyr Val Gly Trp Leu Ala Ala Thr Ala Ala
        355                 360                 365

ctg gcg aag gag acg gcc aca cag gcg agg gca gcg gcg gaa gcg ttt   1152
Leu Ala Lys Glu Thr Ala Thr Gln Ala Arg Ala Ala Ala Glu Ala Phe
    370                 375                 380

ggg acg gcg ttc gcg atg acg gtg cca cca tcc ctc gtc gcg gcc aac   1200
Gly Thr Ala Phe Ala Met Thr Val Pro Pro Ser Leu Val Ala Ala Asn
385                 390                 395                 400

cgc agc cgg ttg atg tcg ctg gtc gcg gcg aac att ctg ggg caa aac   1248
Arg Ser Arg Leu Met Ser Leu Val Ala Ala Asn Ile Leu Gly Gln Asn
                405                 410                 415

agt gcg gcg atc gcg gct acc cag gcc gag tat gcc gaa atg tgg gcc   1296
Ser Ala Ala Ile Ala Ala Thr Gln Ala Glu Tyr Ala Glu Met Trp Ala
            420                 425                 430

caa gac gct gcc gtg atg tac agc tat gag ggg gca tct gcg gcc gcg   1344
Gln Asp Ala Ala Val Met Tyr Ser Tyr Glu Gly Ala Ser Ala Ala Ala
        435                 440                 445

tcg gcg ttg ccg ccg ttc act cca ccc gtg caa ggc acc ggc ccg gcc   1392
Ser Ala Leu Pro Pro Phe Thr Pro Pro Val Gln Gly Thr Gly Pro Ala
    450                 455                 460

ggg ccc gcg gcc gca gcc gcg gcg acc caa gcc gcc ggt gcg ggc gcc   1440
Gly Pro Ala Ala Ala Ala Ala Ala Thr Gln Ala Ala Gly Ala Gly Ala
465                 470                 475                 480

gtt gcg gat gca cag gcg aca ctg gcc cag ctg ccc ccg ggg atc ctg   1488
Val Ala Asp Ala Gln Ala Thr Leu Ala Gln Leu Pro Pro Gly Ile Leu
                485                 490                 495

agc gac att ctg tcc gca ttg gcc gcc aac gct gat ccg ctg aca tcg   1536
Ser Asp Ile Leu Ser Ala Leu Ala Ala Asn Ala Asp Pro Leu Thr Ser
            500                 505                 510

gga ctg ttg ggg atc gcg tcg acc ctc aac ccg caa gtc gga tcc gct   1584
Gly Leu Leu Gly Ile Ala Ser Thr Leu Asn Pro Gln Val Gly Ser Ala
        515                 520                 525

cag ccg ata gtg atc ccc acc ccg ata ggg gaa ttg gac gtg atc gcg   1632
Gln Pro Ile Val Ile Pro Thr Pro Ile Gly Glu Leu Asp Val Ile Ala
    530                 535                 540

ctc tac att gca tcc atc gcg acc ggc agc att gcg ctc gcg atc acg   1680
Leu Tyr Ile Ala Ser Ile Ala Thr Gly Ser Ile Ala Leu Ala Ile Thr
545                 550                 555                 560

aac acg gcc aga ccc tgg cac atc ggc cta tac ggg aac gcc ggc ggg   1728
Asn Thr Ala Arg Pro Trp His Ile Gly Leu Tyr Gly Asn Ala Gly Gly
                565                 570                 575

ctg gga ccg acg cag ggc cat cca ctg agt tcg gcg acc gac gag ccg   1776
Leu Gly Pro Thr Gln Gly His Pro Leu Ser Ser Ala Thr Asp Glu Pro
            580                 585                 590
```

```
gag ccg cac tgg ggc ccc ttc ggg ggc gcg gcg ccg gtg tcc gcg ggc   1824
Glu Pro His Trp Gly Pro Phe Gly Gly Ala Ala Pro Val Ser Ala Gly
        595                 600                 605

gtc ggc cac gca gca tta gtc gga gcg ttg tcg gtg ccg cac agc tgg   1872
Val Gly His Ala Ala Leu Val Gly Ala Leu Ser Val Pro His Ser Trp
    610                 615                 620

acc acg gcc gcc ccg gag atc cag ctc gcc gtt cag gca aca ccc acc   1920
Thr Thr Ala Ala Pro Glu Ile Gln Leu Ala Val Gln Ala Thr Pro Thr
625                 630                 635                 640

ttc agc tcc agc gcc ggc gcc gac ccg acg gcc cta aac ggg atg ccg   1968
Phe Ser Ser Ser Ala Gly Ala Asp Pro Thr Ala Leu Asn Gly Met Pro
                645                 650                 655

gca ggc ctg ctc agc ggg atg gct ttg gcg agc ctg gcc gca cgc ggc   2016
Ala Gly Leu Leu Ser Gly Met Ala Leu Ala Ser Leu Ala Ala Arg Gly
            660                 665                 670

acg acg ggc ggt ggc ggc acc cgt agc ggc acc agc act gac ggc caa   2064
Thr Thr Gly Gly Gly Gly Thr Arg Ser Gly Thr Ser Thr Asp Gly Gln
        675                 680                 685

gag gac ggc cgc aaa ccc ccg gta gtt gtg att aga gag cag ccg ccg   2112
Glu Asp Gly Arg Lys Pro Pro Val Val Val Ile Arg Glu Gln Pro Pro
    690                 695                 700

ccc gga aac ccc ccg cgg taagatttct aaatccatca cactggcggc cgctcgag 2168
Pro Gly Asn Pro Pro Arg
705                 710
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<210> SEQ ID NO:49
<211> 710
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:tetra-fusion protein DPV-MTI-MSL-MTCC2 (designated Mtb71f)

```
His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
  1               5                  10                  15

Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
             20                  25                  30

Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
         35                  40                  45

Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
     50                  55                  60

Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
 65                  70                  75                  80

Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                 85                  90                  95

Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110

Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140
```

```
Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
145                 150                 155                 160

Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
                165                 170                 175

Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
            180                 185                 190

Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
        195                 200                 205

Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
    210                 215                 220

Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225                 230                 235                 240

Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys Val Asn Thr Leu
                245                 250                 255

Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
            260                 265                 270

Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile Met
        275                 280                 285

Asp Phe Gly Leu Leu Pro Pro Glu Val Asn Ser Ser Arg Met Tyr Ser
    290                 295                 300

Gly Pro Gly Pro Glu Ser Met Leu Ala Ala Ala Ala Ala Trp Asp Gly
305                 310                 315                 320

Val Ala Ala Glu Leu Thr Ser Ala Ala Val Ser Tyr Gly Ser Val Val
                325                 330                 335

Ser Thr Leu Ile Val Glu Pro Trp Met Gly Pro Ala Ala Ala Ala Met
            340                 345                 350

Ala Ala Ala Ala Thr Pro Tyr Val Gly Trp Leu Ala Ala Thr Ala Ala
        355                 360                 365

Leu Ala Lys Glu Thr Ala Thr Gln Ala Arg Ala Ala Ala Glu Ala Phe
    370                 375                 380

Gly Thr Ala Phe Ala Met Thr Val Pro Pro Ser Leu Val Ala Ala Asn
385                 390                 395                 400

Arg Ser Arg Leu Met Ser Leu Val Ala Ala Asn Ile Leu Gly Gln Asn
                405                 410                 415

Ser Ala Ala Ile Ala Ala Thr Gln Ala Glu Tyr Ala Glu Met Trp Ala
            420                 425                 430

Gln Asp Ala Ala Val Met Tyr Ser Tyr Glu Gly Ala Ser Ala Ala Ala
        435                 440                 445

Ser Ala Leu Pro Pro Phe Thr Pro Pro Val Gln Gly Thr Gly Pro Ala
    450                 455                 460

Gly Pro Ala Ala Ala Ala Ala Ala Thr Gln Ala Ala Gly Ala Gly Ala
465                 470                 475                 480

Val Ala Asp Ala Gln Ala Thr Leu Ala Gln Leu Pro Pro Gly Ile Leu
                485                 490                 495

Ser Asp Ile Leu Ser Ala Leu Ala Ala Asn Ala Asp Pro Leu Thr Ser
            500                 505                 510
```

Gly Leu Leu Gly Ile Ala Ser Thr Leu Asn Pro Gln Val Gly Ser Ala
515 520 525

Gln Pro Ile Val Ile Pro Thr Pro Ile Gly Glu Leu Asp Val Ile Ala
530 535 540

Leu Tyr Ile Ala Ser Ile Ala Thr Gly Ser Ile Ala Leu Ala Ile Thr
545 550 555 560

Asn Thr Ala Arg Pro Trp His Ile Gly Leu Tyr Gly Asn Ala Gly Gly
565 570 575

Leu Gly Pro Thr Gln Gly His Pro Leu Ser Ser Ala Thr Asp Glu Pro
580 585 590

Glu Pro His Trp Gly Pro Phe Gly Gly Ala Ala Pro Val Ser Ala Gly
595 600 605

Val Gly His Ala Ala Leu Val Gly Ala Leu Ser Val Pro His Ser Trp
610 615 620

Thr Thr Ala Ala Pro Glu Ile Gln Leu Ala Val Gln Ala Thr Pro Thr
625 630 635 640

Phe Ser Ser Ser Ala Gly Ala Asp Pro Thr Ala Leu Asn Gly Met Pro
645 650 655

Ala Gly Leu Leu Ser Gly Met Ala Leu Ala Ser Leu Ala Ala Arg Gly
660 665 670

Thr Thr Gly Gly Gly Gly Thr Arg Ser Gly Thr Ser Thr Asp Gly Gln
675 680 685

Glu Asp Gly Arg Lys Pro Pro Val Val Val Ile Arg Glu Gln Pro Pro
690 695 700

Pro Gly Asn Pro Pro Arg
705 710